ANNOUNCEMENT

American Society for Clinical Nutrition

ON MAY 1, 1960, a new professional society was established—the American Society for Clinical Nutrition. Sixty-nine physicians, members of the American Institute of Nutrition, joined together to form this clinical society whose aims are the following:

(1) to foster high standards of research in human nutrition,

(2) to promote undergraduate and graduate education in human nutrition,

(3) to provide an opportunity for investigators of problems of human nutrition and metabolism to present and discuss their research activities and results, and

(4) to provide a journal for the publication of meritorious work on nutrition in man.

The new society is the clinical section of the American Institute of Nutrition, but will be autonomous in selecting officers, time and location of meetings and in determining publication policies.

The elected officers are President, Richard W. Vilter, M.D., professor of medicine and chairman of the department, University of Cincinnati, College of Medicine; President-Elect, Robert E. Olson, M.D., professor and head of the department of biochemistry and nutrition, University of Pittsburgh, Graduate School of Public Health; Secretary-Treasurer, Robert E. Hodges, M.D., associate professor of medicine, University of Iowa, School of Medicine.

The governing council consists of Willard A. Krehl, M.D., associate professor of medicine, Marquette University, School of Medicine; Robert S. Goodhart, M.D., scientific director,

National Vitamin Foundation; and William B. Bean, M.D., professor of medicine and chairman of the department, University of Iowa, School of Medicine. Editor of the official publication of the society, *The American Journal of Clinical Nutrition*, is S. O. Waife, M.D., associate in medicine, Indiana University Medical Center.

Membership will be limited to physicians (or scientists with doctorates in a basic science) who are actively engaged in research and teaching in the the several areas of human nutrition and who have demonstrated merit and continuing interest in this field as evidenced by publication of original research. Applicants must be approved by the Society as well as the American Institute of Nutrition. Additional information may be obtained from the Secretary-Treasurer, Dr. R. E. Hodges, of Iowa City.

This Journal is honored to have been selected as the official publication of the new society. Several innovations are being contemplated (to begin in 1961) which will make this Journal a more effective medium for the dissemination of scientific information.

The next meeting of the American Society for Clinical Nutrition will take place in May, 1961, in Atlantic City, New Jersey, in conjunction with the American Society for Clinical Investigation.

We believe the formation of this organization marks a significant milestone in the growth and development of the science of clinical nutrition in this country. The formation of the society is propitious at this time when there is heightened recognition of the importance of metabolic

and nutritional derangements to disease with increased interest in prevention thereof through supplementation, with world-wide investigation of a multitude of disorders from kwasiorkor to atherosclerosis and with acceleration of technologic advances. If there ever was a need for scientific approach to problems in a field beset by misguided reformers, uninformed "authorities" and quacks, it is now.

More important, this society by maintaining the highest scientific standards in its meetings and publication will do much to raise the status of scientific nutritionists and to encourage the teaching of the science of medical nutrition as distinct from (though allied with) biochemistry or dietetics. We wish the American Society for Clinical Nutrition good fortune in their courageous enterprise.

Editorial

Vitamin A Nutrition and the Skin

THE means of detecting nutritional de- ficiency in man are primarily three: (1) the measure of intake of the nutrients; (2) evidence of disturbed metabolism and function of the nutrient; and (3) physical changes in the tissues of the body resulting from the deficiency. Of these, the first is predictive and requires final confirmation by the other two. The second is essentially biochemical and physiologic, functional in nature, and in some instances a forerunner and producer of the physical changes comprising the third. Because of the many unknown variables (even in the meticulous study of a single subject) the first is the least accurate and less susceptible to accurate correspondence with the conditions disclosed by the other two meth- ods. Although there are obvious factors which can cause failure of close agreement between the chemical and physical findings, the most obvious of which is the degree of functional change needed to produce physical change, there often are discrepancies which are not only difficult to explain but also interfere greatly with the interpretation of physical signs encountered in the examination of subjects.

One of the best examples of such a situa- tion is in relation to vitamin A. Aside from night blindness, the accessible morphologic and structural changes usually found on physi- cal examination and attributed to vitamin A deficiency are xerosis, xerophthalmia and keratomalacia of the eye, Bitot's spots, and xerosis and follicular hyperkeratosis of the skin. Of these I would like to discuss follicular keratosis and its relation to vitamin A intake and to biochemical findings in respect to its significance as evidence of vitamin A deficiency. Although some little dispute exists as to the meaning of Bitot's spots, this is less in doubt,

and xerophthalmia and keratomalacia are generally accepted as expressions of vitamin A deficiency.

Before discussing the correlation or lack of correlation of follicular hyperkeratosis with biochemical findings in vitamin A deficiency, it is necessary to resolve some of the confusion which exists concerning this lesion itself. Part of the confusion is the result of misunder- standing and misinterpretation of the several terms which are used in the descriptive identi- fication. Depending on the severity and ex- tent of the lesions, the knowledge, experience and ideas of the examiner, the terms used range from xerosis to folliculosis, to keratosis, to follicular keratosis, to perifollicular hyper- keratosis. Other common descriptive terms are "crackled skin," "toadskin," "goose flesh" and phrynoderma.

For the purpose of clarification, let us accept for the present the description and definition of the gross and microscopic lesions of the skin given by Frazier and Hu[1] as characteristic of follicular hyperkeratosis. It is recognized that objections may be raised to the approval of this as an acceptable definition of the lesions under discussion, but this will be primarily due to the occurrence of early or minor degrees or minor modifications or variations of the lesion which have confused the situation. If moderate to well developed changes of the type described by Frazier and Hu are accepted as representing the characteristic lesion, the following discussion will be more clearly de- fined.

Despite the occurrence of these gross and microscopic findings under conditions which Frazier and Hu and later others related to vitamin A deficiency, increasing doubt of such a causal relationship has arisen. The reasons for this are several. In part it is the result of

semantic difficulties and failure of observers and investigators to agree on characterization of the lesion and even the names given to it. In this connection it should be admitted and emphasized that these changes in the skin may result from other causes and, even though they may be caused by vitamin A deficiency, are not specific for it. (On the other hand, this does not mean they cannot result from vitamin A deficiency.)

However, the principal objections to the view that this lesion of the skin is the result of vitamin A lack are (1) failure of positive correlation of the occurrence of this lesion of the skin with apparent levels of vitamin A and carotene intake and the amount of vitamin A and carotene in the blood and vitamin A in the liver and (2) failure, according to some reports, of the lesion to respond to treatment with vitamin A.

There are several explanations which can be offered to refute these arguments against vitamin A deficiency as the cause of the lesions of the skin under consideration, some of which have been well recognized and others which have not. First, as already stated, is the lack of agreement as to just what constitutes the lesion under discussion. Since the lesion can be expected to vary somewhat depending on the degree of severity or stage of the process, some confusion might be expected. Also, as Frazier et al.[2] have shown, the changes in the skin presumably produced by vitamin A deficiency vary with the age of the subject from infancy to adulthood. This, when combined with inexperience in the findings and interpretations of an examination of the skin, makes confusion and disagreement understandable.

Many recent studies have dealt with mild changes in the skin in which there may be some doubt as to the correctness of the diagnosis. In this connection it is worth while pointing out that in many studies in which vitamin A was reported to cause improvement or cure, the follicular hyperkeratosis was moderate to severe, extensive in distribution and presented no doubt as to the real diagnosis. For examples see the photographs of the subjects in the report of Frazier et al.[2]

More recently, it has been argued that the administration of fatty acids themselves are able to relieve the skin changes ascribed to a deficiency of vitamin A and the earlier reports of success with vitamin A have been explained on the basis that at those times vitamin A was available only in an oily menstruum (fish oils).[3] On the other hand, failure to improve or cure the lesions with the administration of fatty acids alone has been reported.[4] In some populations in which follicular hyperkeratosis has been found, the diets have apparently contained a sufficiency of fatty acids.[5]

There are other relations to fat and fatty acids which must be taken into consideration. Fat is well known to influence the absorption of vitamin A and, in particular, carotene. If one takes into consideration the range of severity of the skin lesion from borderline to marked, and the range of intake of vitamin A from barely sufficient to completely insufficient, the possible indirect effect of fat is clearly apparent. The same may be the case with protein, a low intake of which has also been suspected of being a factor in some way leading, in some relationship to vitamin A, to the production of follicular hyperkeratosis. Diets low in protein are notorious in countries in which vitamin A is also deficient.

Poor agreement between the occurrence of perifollicular hyperkeratosis and the intake of vitamin A and its level in the blood is well documented. In particular the recent surveys of populations conducted by the ICNND repeatedly have been shown a general failure of reasonably close correlation of these two criteria of vitamin A nutrition.[6]

There are several explanations of this discrepancy, none of which is convincing as an all-inclusive cause. Interference with the absorption of vitamin A and carotene has been mentioned as a possible explanation for the lack of correspondence between apparent intake and the occurrence of skin lesions. Recently it has been recognized that many carotenoids giving a positive reaction in the chemical tests for carotene are not true precursors of vitamin A and give false values for the intake or blood levels of carotene, and hence vitamin A.

Less frequently appreciated is the time relationship between the course of the skin lesion and the intake or blood levels of the vitamin. The lesion of the skin is a physical, anatomic change. It takes a period of weeks to appear and may take even longer to disappear under treatment or in spontaneous response to natural changes in the diet. On the other hand, the intake of vitamin can vary overnight or, more practically, in a period of days. Similarly the blood level of vitamin A can be increased rather rapidly, and it may be that in instances of borderline storage (on chronically low intakes) the level can drop relatively rapidly. Thus it is entirely possible, theoretically and probably in fact, to have *no* changes in the skin when the intake and blood levels are low, and well defined changes when the former are normal or high. In looking for correlations, these time relationships are important. However, in recent tests of the effect of fats and/or vitamin A on the skin lesions, adequate control of this factor was employed. All in all, when the evidence for and against vitamin A deficiency as a cause of hyperkeratosis follicularis is balanced, it must be concluded that the question remains unsettled.

Until recently the relation of vitamin A to perifollicular hyperkeratosis (and abnormalities of epithelium generally) has been generally thought of only in terms of intake, absorption, body stores (primarily liver) and concentration in the blood. Indeed it has been the failure of the last to correlate with the lesions of the skin that has cast the greatest doubt on vitamin A deficiency as a cause of the latter. Relatively little attention has been paid to the more detailed metabolism of the vitamin, its deposition in tissues other than the liver, its excretion, destruction or transformation into related forms. Neither has the fate of carotene in the body been fully determined.

Actually only a small part of the body store of vitamin A is found elsewhere than in the liver. Despite its apparent important role in the metabolism of the epithelial cells, little can be demonstrated in these tissues. Little is excreted. Some is destroyed in the function of visual adaptation to light. As would be expected from the occurrence of normal or near normal blood levels in patients with skin lesions, biopsies of the liver have revealed a normal concentration and distribution in that organ in patients with keratosis follicularis.[7]

Recently, more attention has been directed to the occurrence of vitamin A and carotene in the skin. Only minute amounts of carotene and vitamin A are found in normal skin and sebum by chemical tests and neither have actually been demonstrated by delicate (sensitive) fluorescent histologic technics.[7] However, Greenberg, Cornbleet and Demovsky,[7] by the intracutaneous injection of a water-solubilized suspension of carotene in the form of carrot oil, have demonstrated with fluorescent microscopy the presence of vitamin A in the sebaceous glands within thirty minutes of the injection; somewhat later it was found in the sebum in the sebaceous duct emptying into the hair follicle, in the depression at the follicle orifice and in a thin layer of the superficial epidermis. Later many yellow gold flecks, presumably carotene, were seen in the sebaceous gland cells which had contained vitamin A, but not in the fatty droplets or in the sebum.

Topical application followed by brisk rubbing resulted in the presence of much smaller amounts of carotene and vitamin A in the respective locations.

These investigators hypothesize that normally the same situation exists; namely, the conversion of carotene to vitamin A in the sebaceous cells and later elsewhere, except that the amount of vitamin A is much less and is not detectable chemically or histologically.

These findings raise the question whether or not the function of vitamin A in the skin operates by a mechanism which is independent of the storage of the vitamin in the liver, its concentration in the blood and its metabolism in other situations. That it is entirely independent of the amount in the plasma seems unlikely if one accepts the benefit apparently obtained when the blood values are low and relatively large doses of vitamin A are given. On the other hand, it is conceivable that the ordinary mode of action is conversion of

carotene to vitamin A *in situ* in the cells of the sebaceous gland under the influence of some other agent (as in the conversion of pro-vitamin D to the active vitamin under the action of ultraviolet light). Under these circumstances it is possible that the lesions of the skin are a manifestation of vitamin A deficiency but mediated through a mechanism involving other factors which at present are unknown.

Recent speculation involving vitamin A in the synthesis of glycogen[7,8] and relating it through glycogen to the mitochondria and the process of mitosis are intriguing in the light of the changes which have been observed experimentally in the skin in a recovery from the lesions of vitamin A deficiency. It takes no stretch of the imagination to link them with the concept of vitamin A's function of pre-serving the health of the cell and not partici-pating in its function. However, such specu-lations should not substitute for an intensified study of the metabolism of vitamin A and carotene in the skin itself.

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Clinical Reports

Food, Nutrition and the Space Traveler

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THE POSSIBILITY of manned space flight in the near future presents striking challenges in the areas of protecting and sustaining man in a new and hostile environment. This paper will primarily consider the problems concerning food, but it will also touch on those problem areas which are closely associated. What will travel, involving great distances in space, mean with respect to food?

When planning a trip into space, we need to know how much time it will take. In our discussion of food, we can probably best examine the problems if we divide space feeding into three classes based on the length of flight. First, there will be short periods of space flight, lasting not more than two or three days. Second, there will be trips of intermediate duration, i.e., those of more than two or three days, extending to several months. Third, there may be long flights or space voyages which may last for years and possibly even for generations sometime in the more distant future.[1]

Why should we consider time periods such as these?

Each of these categories of flight involves specific problems of which food is but one. The problems connected with these three flight durations are related to weight. Many pounds of fuel and oxidizer are required to place a single pound of payload in orbit around the earth. It takes even more to effect a velocity sufficient to escape the earth's gravitational field and attain true flight in space. Weight conservation is, therefore, a highly critical consideration for space flight. Man must have oxygen every minute that he lives, and (when supplied in the most concentrated form as a liquid) he will utilize about $1\frac{1}{2}$ pounds of oxygen per day or 550 pounds per year, provided there is no leakage or other loss. Hence, the logistics of carrying adequate supplies of oxygen in a space vehicle soon become critical. Another of man's needs, one which is more closely related to food, is his requirement for water. The average man engaged in light occupation needs approximately 2,200 ml. of water per day, including the liquids consumed and water contained in food. In terms of weight, this requirement involves about 5 pounds per day, an amount approaching 1 ton per year. Therefore, weight becomes a limiting factor in space flights.

When a person eats all of his meals at home, approximately 7 pounds of food are consumed daily. This includes the weight of inedible portions of food, food lost in preparation and some materials used in packaging the food. In addition, numerous items are needed for food storage, preparation and service. Obviously, even for short periods of flight into space, food such as that used at home would involve excessive loads of weight. Stored food, then, is a third limiting factor.

What can be done about these three restrictive factors?

During two to three day flights, it will be economical to carry liquid oxygen supplies, stored water and ready-to-eat foods comparable to those now used in air travel. However, this will not be true for missions of longer
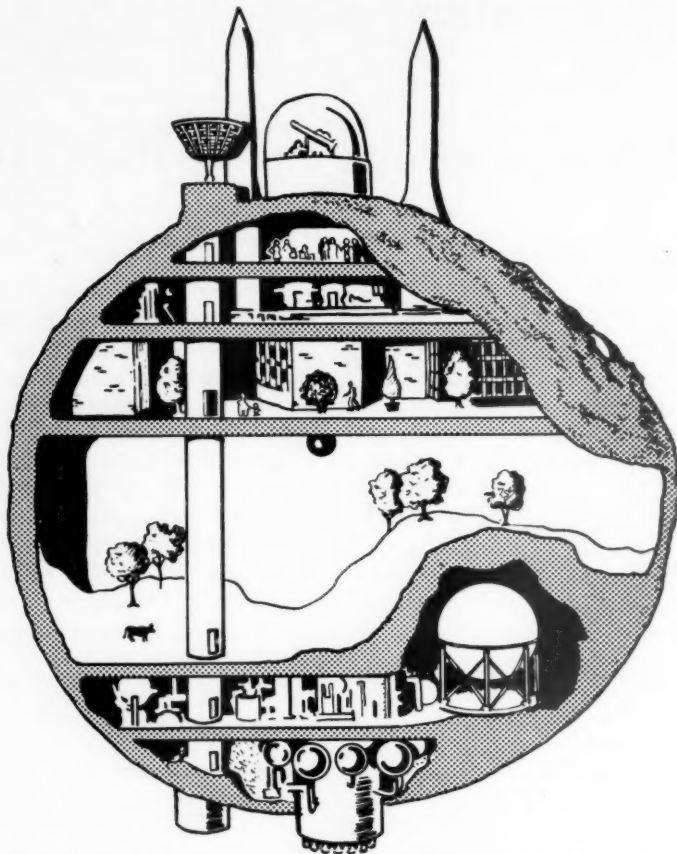
FIG. 1. This is a model of an asteroid space ship, constructed from the remains of a planet which broke up perhaps several billion years ago. A vehicle such as this might contain everything that fifteen generations of space voyagers would need during their trip. (Space Ark Model, Courtesy of the Hanover Bank, New York.)

duration. In space travel lasting more than three days (but only for comparatively limited periods of time), weight problems can be overcome by utilizing our present technology. Briefly, by utilizing a source of energy, the atmosphere within a space ship can be controlled. Carbon dioxide can be broken down into its constituent parts and the released oxygen can be reutilized. Water can be recovered from the atmosphere. It can also be reclaimed from body wastes, then purified and made safe for use.

Great savings in weight of food can be effected by using concentrated and dehydrated foods. Of necessity, these will be the "quick-serve" type, packaged in lightweight containers from which the food can be eaten. Equipment for the preparation of food can be limited to that for heating food. With such a feeding program, one can estimate that the former requirement of 7 pounds of packaged food can be reduced to half that quantity for travel in space. Thus, our weight problems can be solved for a period of many days or until the weight of stored food becomes too great a penalty.

This period of many days is difficult to define with any degree of precision. At this time it

remains unknown or a value of **X**. However, for the purpose of discussion, let us estimate that $3\frac{1}{2}$ pounds of packaged concentrated and quick-serve foods per day will provide a man with adequate nutrition. From these foods, he will receive approximately 3,500 calories with the usual distribution of protein, fat and carbohydrate, and the necessary minerals and vitamins. This kind of feeding will involve about 1,300 pounds of food per man per year of flight. The provision of a smaller number of calories would involve somewhat less weight; however, the storage of amounts of food of such magnitude for long flights in space is unrealistic. Guess limits this **X** time value to two to six months; i.e., it might be feasible to carry a supply of dehydrated and concentrated foods for a two to six month period. A space voyage of longer than six months would very probably exact too great a weight penalty.

Once it is no longer economical to transport food, regeneration of carbon and nitrogen compounds will be required. Wastes must be converted into food. Such a solution is related to the provision of oxygen for breathing. While plants produce oxygen, they also produce food for man and food for the animal life which becomes food for man. It is in this way that we get all of our oxygen and food on earth. Nature recycles water and regenerates carbon and nitrogen compounds. This regeneration has been going on for millions of years in the earth's great expanses of land, air and water. A practical means to regenerate the carbon and nitrogen compounds man needs will remove all food limitations to the duration of space flight. Certainly, to install such a system in a space ship, the natural processes must be speeded up and the apparatus condensed into a small space and restricted in weight. The technical feasibility of such regenerative cycling is not in question for it is well established in nature. Basic research in the strict sense of the word is not required; but applied research and development are needed. Processes which take place on the earth to purify the atmosphere and produce our foods must be synthesized, using some form of energy in a closed and balanced ecologic system which can be carried aboard a

TABLE I

Inventory of Food Used in the Manhigh III Balloon Trial

Food	Inventory of Food	
	Unit	Amount Provided
Beverages		
Coffee drink*	8 oz. bottle	1
Milk, whole	8 oz. can	2
Chocolate drink	8 oz. can	2
Candy		
Chewing gum	Package	1
Chocolate	2 oz. package	1
Cake, cookies, crackers		
Chocolate cookies	Package of 5	1
Pound cake	$1\frac{1}{2}$ oz. carton	1
Crackers	Package of 5	1
Fruit and juices		
Raisins	$1\frac{1}{2}$ oz. carton	1
Orange juice	$5\frac{1}{2}$ oz. can	3
Pineapple juice	6 oz. can	3
Meat		
Beef cubes	3 oz. package	3
Beef, semisolid	$3\frac{1}{2}$ oz. tube	2
Chicken, semisolid	$3\frac{1}{2}$ oz. tube	2
Nuts		
Assorted	5 oz. package	1

* A high protein beverage prepared with a protein supplement.

space vehicle (Fig. 1). Actually some progress toward a solution has been made. Algae in the presence of light absorb carbon dioxide and liberate oxygen. Work is currently under way to find or produce a more efficient form of algae. In addition, there has been considerable speculation about the possibilities of algae as a source of food.[1]

Man's first space rides will be short, perhaps a few hours. During these journeys he will orbit the earth at comparatively low altitudes of a few hundred miles. Flights may not last even long enough to require a meal. During longer journeys, however, he will need food At present, our interest is concentrated on these longer flights. During initial ventures into space which are of sufficient duration to require food, the astronaut will travel in a sealed cabin. His movement will be restricted and many protective measures will be employed. These protective measures together with the acceleration forces involved in take-off, transformation
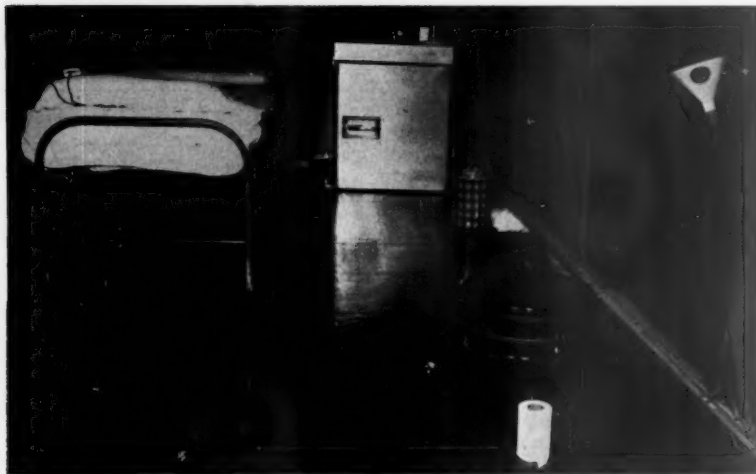
FIG. 2.   The chamber depicted above was used in our study of men in dark isolation.

to a state of weightlessness, heat, vibration, noise, the doubts which accompany exploration of the unknown, and a keen desire to return to earth with as much data as possible will subject the astronaut to tremendous stresses, both physical and psychologic.   It will be one of the functions of food to alleviate these stresses insofar as possible rather than to further them. Food can readily assume this role of alleviation of stress, since it will be one of few pleasurable acts and forms of activity associated with man's existence on earth.   The importance of the role of food to man in a situation of stress has already been demonstrated in high altitude balloon flights and in studies of isolation.

The Air Force, in its endeavors to explore and anticipate the problems of manned satellite flight, has performed a series of high altitude balloon flights.[2]   These manned balloons when flown at altitudes of approximately 100,000 feet provide conditions equivalent to those in space.   Hence, they have served as tests for many physiologic and psychologic aspects of space flight.   The role of nutrition has been satisfactorily accomplished in these balloon flights from the standpoints of food acceptability and adequacy.   (Table 1 provides a sample inventory of food used in the balloon flights.)   In each flight, food has assumed a significant emotional and social role.   One pilot indicated that candy and nuts were consumed during the descent portion of the flight.   These he considered as a reward for a difficult job which was near completion. Another pilot referred to pound cake as, "A very welcome sight.   It appeared as a Christmas gift and gave me a lift in spirit."

Additional data relative to the role of food in a situation of stress have been obtained from isolation studies conducted at the Aerospace Medical Division.[3]   In these tests the subject is isolated from some of the usual sources of sensory stimulation, i.e., sound and light. The person's ability to adapt to this unusual situation is studied.   Sensory deprivation is achieved by placing a subject in a dark sound-proof chamber (Fig. 2).   The chamber is furnished with a bed, chair, refrigerator and chemical toilet.   The isolated subject is studied to determine how he reacts and the means he employs to defend himself against the effects of isolation.   In order to study these factors prolonged periods in isolation are necessary. Hence, feeding becomes essential.

Different kinds of food are provided in advance.   Those which can be distinguished by their own shapes, such as eggs or triangles of processed cheese, are wrapped in aluminum foil.   Those which require packaging are put into plastic containers.   Each food group is packaged in a differently shaped container. Within a specific group, the containers of

Fig. 3. The above supply of food is included in the dark isolation chamber.

Fig. 4. The system of coding various groups of food used in our study.

different foods are coded with masking tape to facilitate identification. The subject is apprised of the code system used for identifying the foods before entering the chamber. He also is indoctrinated as to the arrangement and placement of food, both in and outside of the refrigerator (Figs. 3 and 4). Dark isolation trials have ranged from six to one hundred and sixty-eight hours. The significance of food during these trials has varied. Some subjects have spent excessive amounts of time eating, nibbling or counting food; others have become angry at the food or fond of it. Evidence is strong that food is used as a tool to obtain personal satisfactions.

Application of data obtained from manned high altitude balloon flights and isolation studies indicate that palatability, acceptability and ease of manipulation of food will be extremely important facets of feeding man in space travel. Therefore, consideration, insofar as possible, of the food likes and dislikes of each person engaged in space travel is deemed essential. In addition, each subject should be given ample opportunity to become familiar with the foods that are available and with their specialized packages.

How will the astronaut eat? What will he eat? When will he eat? These are problems of a practical nature. The answer to the first question is comparatively simple. The environment of the cabin will permit facile intake of food and drink except during the period of launch and during re-entry into the earth's atmosphere. However, weightlessness, in conjunction with many necessary protective measures, would make the conventional handling and eating of food somewhat difficult and awkward. Eating from a plate with knife, fork and spoon will not be possible. A number of interesting phenomena would occur when ordinary methods of eating and drinking are employed in a space ship in a state of weightlessness. If a piece of meat should slip while being cut, it would fly off the plate and splatter against the wall, bounce back and then continue to bounce back and forth off the walls, ceiling and floor. A fork full of peas raised to the mouth would continue in its upward flight to the ceiling and be reflected back, bombarding like buckshot. A cup of coffee raised to the mouth would result in the astronaut's receiving the contents in his face. To avoid these frustrating experiences, it is necessary to confine foods and liquids in containers from which they can be squeezed into the mouth (Fig. 5). In lieu of the usual utensils, liquids and semisolids can easily be served from collapsible "squeeze" tubes. Solids in bite size form can be removed by hand from a covered container and placed directly into the mouth.

Fɪɢ. 5. Liquids and semisolids for the space traveler are packaged in collapsible "squeeze" tubes as illustrated.

From our limited knowledge to date, no real problems, involving the chewing or swallowing of food in a weightless state, are anticipated.[4] However, we are actually in no position to judge man's ability to eat under prolonged conditions of weightlessness. At present, we can simulate weightlessness for only about a minute at a time by flying an aircraft in a parabolic flight path. This is, of course, not long enough to gather valid information concerning the mechanics of eating, ease of digestion, absorption and nutritional requirements. During prolonged weightlessness, it is conceivable that man's energy requirements will decrease, since he will not be combating the stress of gravity as he does on earth.

What will the astronaut eat? The initial flights will necessitate controlled feeding for a seventy-two hour period prior to the flights. Protective clothing will preclude defecation during flight. To minimize this problem, a diet high in protein and low in residue is recommended. A sample day's menu is as follows: breakfast consisted of orange juice, cream of wheat, sugar, nutmeg, scrambled eggs, Canadian bacon, toast, butter, jelly, coffee; lunch consisted of chicken noodle soup, veal cutlet, buttered noodles, bread, butter, canned pears, coffee or tea; and dinner consisted of tomato juice, baked chicken, steamed rice, pureed peas, hard roll, butter, lemon sherbet,

cookies, coffee or tea. This diet provides foods which are almost completely absorbed from the gastrointestinal tract, thereby leaving a minimum of bulk for the formation of feces. Such a diet, already tested on numerous subjects, has proven acceptable and effective.

Preflight feeding recommendations also include a low fat meal, which has a high content of carbohydrate and a moderate content of protein, immediately prior to take-off. A meal of this type aids man's tolerance to increases in altitude. Foods now envisioned for use during initial flights will consist of a variety of sandwiches, meats such as beef, turkey and ham, fruit sauces, fruit juice, chocolate drink, cookies and candy. Solid foods will be bite size and liquids and semisolids will be packaged in collapsible tubes. In addition, 3,000 ml. of water will be provided for each day of flight. This amount, in addition to providing an adequate amount of liquid, will serve as a reserve supply if needed, following return to earth. Consumption of a multivitamin preparation during flight will probably be practiced as there are experimental data which indicate the beneficial effects of the water-soluble vitamins during stress.

When man is subjected to the removal of the normal force of gravity for short periods of time, he may show tendencies toward disorientation, discomfort, nausea and motion sickness.[5]

TABLE II
Liquid Diets

Menu A	Menu B
Breakfast	Breakfast
Tomato juice	Orange juice
Coffee drink	Coffee drink
Midmorning	Midmorning
Apple juice	Pineapple juice
Lunch	Lunch
Chicken drink	Chicken bisque
Chocolate milk	Chocolate milk
Midafternoon	Midafternoon
Apricot nectar	Apple juice
Dinner	Dinner
Beef drink	Vegetable soup
Lemon drink	Lemon drink
Coffee drink	Coffee drink
Midevening	Midevening
Chocolate milk	Chocolate milk

Should these reactions occur during prolonged periods of weightlessness, deleterious effects could be minimized if the food provided were in the more easily swallowed form of liquids. Therefore, a liquid diet was formulated for a person engaged in sedentary activity and evaluated at the Aerospace Medical Division. This diet has a high content of protein and contains an adequate but minimum amount of calories and sufficient nutrients.

Fifteen men, ranging in age from twenty-one to twenty-nine years, participated in this study for a five day period while performing their usual activities in the laboratory. Consumption of food was controlled by serving meals, consisting of two or three beverages, in the nutrition laboratory. Two daily menus, as described in Table II, were used alternately. Fruit juices and a chocolate drink were made available as between-meal snacks. Each day's meals provided approximately 2,500 calories and 100 gm. of protein. This amount of protein was obtained by using protein supplements, strained meats and a concentrated ice cream mix. The average daily food intake approximated 2,300 calories and 100 gm. of protein. Changes in weight were insignificant, indicating that the caloric intake was adequate for the activity performed. The types of gastrointestinal disturbances reported were minor; none of the subjects curtailed their food intake or were in any way incapacitated because of these disturbances. Psychologically, no adverse effects were noted. The morale of all subjects was good throughout the test period.

Average preference ratings for foods revealed a need to classify the foods of the diet into two groups: those which are commercially available and are familiar items and those which are prepared in the laboratory and have new and unfamiliar tastes. Foods in the first group were well liked, whereas unfamiliar foods were initially disliked. However, as the test progressed and the subjects became familiar with these foods, preference ratings increased. Data indicate the feasibility of using a liquid diet which has a high content of protein for initial space flights if a diet of this consistency is required.

When will the astronaut eat? Consumption of food at two or three hour intervals is considered necessary to insure an optimum state of nutrition at all times. It is most likely that the stresses inherent in space flight will interfere with appetite. Changes in day and night cycling while in orbit as well as adaptation to a state of weightlessness may diminish the desire for food. If the astronaut regulates his intake according to his desire for food and fluid, an emergency could arise at a time when the subject's state of hydration and nutrition are already inadequate. This occurred in an instance of stress from extreme heat which was sudden and unexpected during a manned balloon flight at a high altitude.[2] Data from this experience suggest the need for frequent scheduled consumption of food during all phases of unusually demanding missions.

SUMMARY

Current concepts of feeding during space travel are based on (1) our knowledge of aerodynamic flight feeding requirements, (2) the experiences of crews who have flown high altitude, high performance aircraft and those who have made high altitude balloon flights, and (3) the results of a variety of laboratory studies.[7-9] In spite of the wealth of information obtained from these sources, plans for feeding man in space have been designed with-

out knowledge of the stresses produced by confinement in a sealed cockpit while traveling in space. A final determination of nutritional requirements and feeding needs will come only when man goes into space and returns safely to earth.

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The Significance of the History of Goiter
for the Etiology of the Disease

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This discussion is concerned with *endemic* goiter. Tumors of the thyroid may be expected to occur about as frequently as do tumors of other organs. However, thyroid tumors occur much more frequently and this prevalence is conspicuous in certain regions. In many places, endemic goiter appeared quite recently and its subsequent history resembled that of a newly introduced infectious disease.

I am not so naive as to believe that absence of mention means absence of the disease. Only when the description of a population is so detailed as to physique, diseases and medical practices as to make it almost certain that goiter, if present, would have been recorded, do I regard the absence of such mention as an indication of absence. In a few instances, definite statements that the disease was not present, or had not been present until a comparatively short time earlier, are made.

It is impossible to review all the work of fifteen years in this report; only a few instances can be discussed.†

In contrast to the prolonged controversy over the pre-Columbian existence of syphilis, the question of the presence of goiter in the Americas attracted no attention until I raised it. This neglect seems to have been due to two factors. The first was the general acceptance of the view that the disorder was due to some factor in the environment, which was supposed

not to have changed. In this way, Stone was led to the statement "In certain areas, goiter and urinary calculi were endemic as they are today among both their white and red inhabitants." Upon inquiry for the authority for this statement, he replied "Goiter was not noted by the early explorers or pioneers."[1]

The second important cause for the general belief that goiter existed in pre-Columbian America is a curious error in translation, copied from author to author for four hundred years without reference to the original statement. This will be discussed subsequently.

When Europeans first came to the New World, they encountered a new race of men who aroused their curiosity and interest. Long and detailed descriptions of these new men, their physique, mode of life, diseases and methods of treating them, were recorded.

CANADA

In the North our main source of information regarding the Indians of the Great Lakes Region and the St. Lawrence Valley is in the writings of the early missionaries. Some of these were lengthy and have been printed and reprinted individually. Hundreds of others have been collected and published in the original French or Italian and in English translation.[2] The only reference to goiter in these writings was made by Bressionis in 1653, more than a century after the arrival of the French. Bressionis was stationed in the peninsula of Ontario, between Lakes Erie and Huron. (In later years this region of Canada became one in which goiter was prevalent.) He wrote of the Indians in his care: "They are not dark, especially in their youth; they are strong, tall in stature, and well-proportioned; more healthy than we, not even know-

ing the names of many diseases common in Europe such as the stone, gout, rupture, etc. They are not found either hunch-backed or dwarfed or very corpulent, or with goitre."

The first indication of the existence of goiter in Canada is in a manuscript dictionary* of the Algonquin language, which was probably compiled about 1690. The word seems to be a translation of "grosse-gorge" (thick neck), the common word for goiter among the French Canadians. The first definite statement was made by Boucault about forty years later.[3]

The next area of concern is in Western Canada in what is today the city of Edmonton, Alberta. Before there was any permanent settlement in that region, it was visited by five trappers and explorers who left us descriptions of the Indians.[4-7] None of these men-tioned goiter. In 1820, only ten years after the first permanent settlement, Edmonton was visited by John Richardson, the physician who accompanied Sir John Franklin's first Arctic exploration expedition. Richardson's descrip-tion of goiter and cretinism was incorporated in the book by Franklin.[8] Richardson specu-lated as to the cause of these disorders and stated that they were not present at Fort Carlton "although they drink the same waters," that of the North Branch of the Saskatchewan River. In 1841 Simpson[9] wrote that goiter was present in the population along both branches of the Saskatchewan, at least as far as the Forks. This is well below Fort Carlton, where Richardson had said that it was absent some twenty years earlier.

During the subsequent twenty years, every-one who left a record of his visit to Edmonton, with one exception, noted the presence of goiter. In 1858 Hector,[10] a physician who accompanied the Palliser expedition for surveying a route for a railroad, wrote: "I have seen only one case in which there is any approach to cre-tinism." Goiter was prevalent at both Edmon-ton and Rocky Mountain House and Hector made what may be a very significant observa-tion: "The only curious feature seems to be that children born at one fort are never attacked

till removed to the other, and it again disap-pears on their return to their native place." In 1863 it was again mentioned by Milton and Cheadle.[11]

For the next fifty years there were no reports of goiter anywhere in the vicinity. In 1919 the people of the province were disturbed by the prevalence of goiter and especially by one person who preached the danger of this condi-tion, stating that in another generation there would be many idiots. The Dominion authori-ties were asked for help and Shepherd of McGill University was sent. In his report[12] he stated that the goiter was no more prevalent in Alberta than it was in eastern Canada and that it had been common in Three Rivers, Quebec, for generations without deterioration of the people. He added a statement by a Dr. Whitelaw, who had been in Edmonton for nineteen years, the first ten in general practice and the remaining nine as Health Officer. "I am personally acquainted with a great many old timers, who were here when I came here nineteen years ago, and I cannot recol-lect seeing a single case among them."

UNITED STATES

The first mention of goiter in the territory of the present United States appears to be in letters written by a Moravian missionary, quoted by Barton,[13] mentioning its presence among the Delaware Indians in Eastern Ohio in 1797. At about the same time another Moravian missionary, Zeisberger,[14] compiled a dictionary of the Delaware Indians' language. The word for the English "wen" was "knop," which is not Delaware Indian or any other Indian language. It is the German "kropf," (wen or goiter) which the Indians did not pronounce correctly. This is, I believe, almost conclusive evidence that goiter was non-existent among these Indians until the Mora-vians came among them.

In the city of Pittsburgh, goiter seems not to have been known during the period of French occupancy (1752–1765). Within fifty years, it appeared, affected more than 10 per cent of the population or almost every family, and diminished so that there were no new cases whatever.[15]

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CENTRAL AMERICA

The Indians of Mexico, Central and South America excited the interest of Europeans even more than did those of North America. The latter became acquainted, not only with a new race of men, but also with new civilizations which, in many respects, rivaled that of Europe. Some of the Spaniards and their German companions were indeed interested only in gold, but others, especially the missionaries, were interested in the people. They have left us a copious literature.

I have found no certain mention of goiter in Mexico before the nineteenth century. (In 1945 I assumed "la plus vilaine gorge du monde" to mean goiter, but a re-examination of the text indicates that a correct translation is "the ugliest breasts in the world.")[16]

In what is now Guatemala, goiter was first reported *in one place* more than a century after the Conquest. In the nineteenth century, it was reported from several places in Mexico and Central America, many of them at, or near, the coast. Noteworthy are several statements that goiter was not frequent in the highlands in Guatemala or in Mexico.[1]

SOUTH AMERICA

Quesada, the leading conqueror of what is now Colombia, wrote of the Chibcha Indians: "They were the finest people he had seen in the Indies: the men well-formed and strong; the women handsome."[17] Apparently, goiter was present in one valley about fifty years after the Conquest.[18] According to Humboldt[19] Bogota was free from the disease until about 1790. Later, goiter became very common, then it diminished and increased again.[20]

The third and probably the greatest of the pre-Hispanic civilizations was that of the Incas. Their empire embraced not only what is now Peru but also Ecuador, much of Bolivia, northern Chile and Argentina. In 1609 Garcillasso de la Vega, a descendant of the Incas and familiar with their records, wrote his "Los Comentarios Reales de los Incas" (The Royal Commentaries of the Incas). This was translated into English by Sir Paul Rycaut.[21] A passage from the translation reads

as follows: "From Cassamarquilla the Inca proceeded to another People called Papamarca, from the Papas or Dewlaps, which are great bunches that hang from their throats."

This statement was quoted by Barton,[13] from him by Hirsch[22] and from the latter by many others. Garcillasso's book has since been translated by Sir Clements Markham,[23] whose version of this passage is: "From Cassamarquilla the Inca advanced to another important village called Papamarca, which means the village of potatoes because they grow very large there."

I have verified this by reference to the original.[24] Rycaut had confused papa meaning potato with papo meaning dewlaps, double chin, etc. (Papo later became the word for goiter in those parts of Spain in which the disease was prevalent.) This grotesque error is still being repeated, even by a Peruvian professor of the history of medicine, who gave, in Spanish, not Garcillasso's words but a translation into Spanish of Barton's repetition of this grotesque error.[25]

The first definite report[26] of the presence of goiter is dated 1638 and it clearly states: "Solo en las tierras del Cuzco y Chuquisaca hay alguna aqua que a tal, o tal persona crian hinchazones en las gargantas, que llaman cotos." (Only in the lands of Cuzco and of Chuquisaca (now Sucre, Bolivia) is there water which produces swelling in the throat called Cotos.)

"Coto" is an Indian word which, in the sixteenth century, meant heap or protuberance. An instance of its use to mean "mountain" is in "Cotopaxi" (shining mountain). It was later applied to any swelling in the throat, notably parotiditis, and, still later, to goiter.[27]

Previously[1,27] I have shown that the claim that goiter was present before the Spaniards came is without foundation. The disease spread during the seventeenth, eighteenth and nineteenth centuries. A striking feature of the disorder in both Peru and Argentina is that for many years it was accompanied by deaf-mutism not cretinism. Even later, 1914 in Argentina and 1940 in Peru, the proportion of deaf-mutes to cretins or to persons with goiter far exceeded that ever reported elsewhere

except in a few small areas and populations. Diminution in the prevalence of goiter was noted at Salta (Argentina) in 1805 and at Mendoza (Argentina) in 1870. The censuses of Argentina in 1869 and 1895 show that this diminution was general. By the time the third census was taken in 1914, the occurrence of goiter had diminished to such an extent that no separate enumeration was made. The number of deaf-mutes had continued to decrease.[28]

The first mention of goiter in Chile dates from 1814. In 1820 it was still not common. Thirty years later, it was prevalent in and around Santiago but was regarded by the people as a recent affliction. It has spread to other parts of the country but, apparently, is not accompanied by any considerable proportion of deaf-mutism.[27]

AFRICA

A few years ago, a review opened with the statement that goiters were depicted in the sculptures and wall paintings of ancient Egypt. Upon inquiring for evidence for the statement, the author replied that he had none and that he would be perfectly willing to accept my statement (made five years earlier) that there was no evidence for the presence of goiter in ancient Egypt. At the beginning of the nineteenth century, there were several reports describing the diseases of the country but they did not mention goiter. In 1812 it was said that goiter was hardly ever seen in Egypt. Even later reports gave no indication of its presence, and in 1847 it was said that the disease was not endemic. By the end of the century, the occurrence of goiter was more frequent, and by 1919, it was "extremely common."[29]

According to Krueger and Armattoe,[30] goiter was common among the Ewe people of the Gold Coast and Togoland in 1898. Indeed it was the most common surgical complaint. By 1948 there was none.

For several years, the Department of Health of the Union of South Africa[31] had made inquiries regarding the prevalence of goiter. Sporadic cases were found but there was no endemic prevalence. Suddenly in 1927 it was found to be common in "certain remote

localities in the Uniondale district . . . in the case of the Hoeree Valley it was estimated that about 65 per cent of the total inhabitants show definite signs of thyroid enlargement." Years were spent in the analysis of foods with the result "that no fixed relationship could be established between the iodine content of foodstuffs and the incidence of goitre." Here was an almost ideal situation for a careful epidemiologic study. Unfortunately, the opportunity was neglected.*

The report quoted in the preceding paragraph also included the following statement: "The disease is not common in the Union, but other small isolated groups of cases are believed or suspected to exist in the Pretoria district and one or two other localities." By 1948 "it was a serious problem" and in 1955 it was stated that "Schneider speaks rightly of a goitre-belt running from the Mahusa area through the Eastern Transvaal, Swaziland and Natal to George District in the Cape Province."[35]

EUROPE

Switzerland has long had an unenviable reputation for its goiters and cretins. In the opening address to the First International Goitre Conference, Carriere[36] pointed out that "the huge goitres that formerly struck the observer have nearly disappeared." He also called attention to the change in the proportion of registrants for military service who were rejected because of goiter from 10 per cent in 1885 to 2.9 per cent in 1912 and to about 1.3 per cent in 1924 to 1925. As a matter of fact, the diminution in the prevalence of cretinism had been noted as early as 1800[37] and that of goiter before 1875.[38]

In most of Spain, goiter is a recent phenomenon. Nevertheless, it is already disappearing spontaneously in several places, while appearing in others, apparently for the first time.[39]

In France, goiter and cretinism had been known for centuries. However, by 1820 they were reported to be diminishing in the

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*This is not the place for criticism of the evidence adduced in support of the "iodine-lack" hypothesis. This may be found in other publications.[32-34]

Pyrenees and, a few years later, in other parts of France.[22] The diminution continued, and by 1934 the diseases occurred only in certain villages, hamlets and families.[40]

In *The Tempest*, Shakespeare has Gonzalo say (Act III, Scene III):

Faith, sir, you need not fear, when we were boys,

Who would believe that there were mountaineers

Dew-lapped like bulls, whose throats had hanging at 'em

Wallets of flesh? or that there were such men

Whose heads stood in there breasts? which now we find

Each putter-out of five for one will bring us Good warrant of . . .

The phrase "putter-out of five for one" refers to an inverted form of life insurance employed by travelers at that time. "Mountaineers dew-lapped like bulls" is generally regarded as a reference to travelers' reports of goiters, seen in the Alpine regions of Europe. The entire passage clearly indicates that goiters, large ones at any rate, were not known in England in Shakespeare's day. Examination of the published writings of English visitors to Switzerland and the adjoining countries from Shakespeare's time through the eighteenth century, clearly indicates that the large goiters (or anything resembling them) seen in the Alps were unknown to them in England.

The Oxford Dictionary gives the history of every English word that has been used for goiter. The earliest quotation that indicates the prevalence of the disease in England is "Derbyshire neck," attributed to Prosser.[41] I have found it in a medical dictionary of 1743.[42]

In 1700 Charles Leigh, a physician, published his "The Natural History of Lancashire, Cheshire and the Peak in Derbyshire."[43] There were fifty pages devoted to "Distempers of the Region," but there was no mention of goiter or of "Derbyshire neck" or of anything resembling this condition.

It may seem strange that, in the space of forty-three years, goiter should have changed from an apparently unknown condition to one so prevalent as to acquire the name "Derbyshire neck." But we have seen how goiter appeared in Pittsburgh and in Edmonton, became prevalent and then practically disappeared in approximately the same number of years.

From Derbyshire, we can trace the gradual spread of the endemicity of goiter throughout Britain. Why did it appear first in Derbyshire? After more than two hundred years, it is impossible to be certain. However, we know that in 1717 John Lombe of Derby went to Italy to learn the art of spinning a certain kind of silk yarn. He brought back with him two natives of Piedmont, a notorious goiter center. Is it not possible that these two introduced goiter into England?[1]

A somewhat similar situation, this time involving several Germans, occurred in Sweden. There, goiter was first reported from Falun and became known as Faluknölen or Falun lumps. It is perfectly clear that the disease was not common in Falun or anywhere in Sweden before the middle of the eighteenth century. Later it became more frequent in places other than Falun, just as it has become more common, at least in children, in parts of England other than Derbyshire. Certainly, in neither Falun nor in Derbyshire is it now as prevalent as it was formerly.[44]

The history of goiter in many other regions is the same. It was not known in the Philippine Islands[45] or in Ceylon[46] until long after the coming of the Europeans. We have three positive statements that the Maori of New Zealand did not have goiter as late as 1855.[47]

I now turn to the occurrence of goiter, not in a particular region, but among a peculiar people living in different localities.

GOITER AMONG JEWS

The only reference to the endemicity of goiter in Central Spain before the nineteenth century is to be found, not in Spanish, or in Latin, but in Hebrew! In 1492 Isaac Caro was expelled from Spain and in 1497 from Portugal. In Constantinople, he wrote a book in which he stated that diseases were due to one of the following three agents: (1) heredity, (2) association and fraternization and (3) place or climate, owing to the fact that the water or the air is bad, e.g., the town Buitrago, where

"zephek" occurs among people, which is a hard swelling on the "garon" (neck or throat).[48]

The identity of the Hebrew consonants with Buitrago seems certain. The word zephek means the crop of a bird and, in using it to denote a swelling in the throat, Caro was doing what people in other parts of the world have done: for example, the German kropf and the Italian gozzo. Probably, Caro followed the Italian. He did not say that the Jews had or did not have goiter. Apparently, both Jew and Gentile were affected.

It is noteworthy that Eliezer ben Yehudah[49] in compiling his Thesaurus, the basis of modern Hebrew, also used zephek for goiter and gave Isaac Caro as his sole authority. This, in spite of the vast Hebrew literature of more than a thousand years, of which Eliezer was a profound student. Apparently, goiter was rather rare among Jews.

There is other evidence to this effect. In 1776 Read found that, although the incidence of goiter in the city of Metz was great, only one woman was so afflicted in the Jewish quarter. A similar state of affairs, in the same city, was reported in 1827. Indeed, according to a third author, the Academy of Medicine at about that time had raised the question "Why are Jewish women free from goitre?"[39]

The freedom was certainly not absolute and not due to race or to dietary practices. In 1825 another Frenchman found seventy goiters among the 1,770 Jews in the ghetto of Turin and more than a century later, we were told that goiter had been common among Jews in Bukowina for several generations.[39]

COMMENTS

What is the significance of this history for the etiology of the disease? I submit that the history resembles that of an infectious disease, which comes and goes and, sometimes, comes again. Is it not possible that we stand today where we stood a century ago with respect to tuberculosis? Food, environment and heredity all had a part in determining susceptibility, or resistance, to tuberculosis. But the disease was infectious. Is it not possible that the variations in the rate of onset, in the proportion of accompanying cretinism, deaf-mutism and

hyperthyroidism, and in the occurrence in infants and in animals (affecting different species in different regions) are due to differences in the strains of the infectious agent? May not the geographic localization be due to the participation, perhaps necessary, of a vector of limited range? May I recall the history of verruga peruana? This disease, too, was associated with the water of certain streams and its true cause remained unknown for four hundred years after the Europeans came to Peru. Then it was discovered that the disease was caused by a microorganism which was carried by a sand-fly.

Perhaps goiter resembles modern leprosy in that infection ordinarily requires prolonged exposure. Perhaps, in much of the world at the present time, goiter is like herpes simplex; something with which we are all infected, with the actual development of the lesion depending on extraneous factors. Perhaps, it is quite different from either of these.

Would it not be well to explore again along the path opened by McCarrison many years ago?

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Effect of Mixed Fat Formula Feeding on Serum Cholesterol Level in Man

II. Further Study Utilizing a Twenty Per Cent Fat Formula

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Studies of animals by Hegsted et al.[1] have demonstrated the ability of linoleic acid in combination with saturated fatty acids to alter levels of cholesterol in the serum. Extension of this observation into the area of clinical investigation of human subjects has also been reported,[2,3] and the results have been so consistent that Keys and his associates[4] have formulated an equation predicting the effect on levels of cholesterol in the serum of various proportions of linoleic acid and saturated fatty acids. These studies indicate that slightly more than 2 gm. of linoleic acid are needed to counter the effects of 1 gm. of saturated fatty acid. The results differ in studies of rats in which a mixture of equal amounts of saturated and unsaturated fatty acids produced the greatest lowering of serum cholesterol levels.

Clinical extension of these studies has been undertaken by our group in terms of formula type feedings as initiated by Ahrens et al.,[2] incorporating varying levels of saturated and unsaturated fatty acids in the form of coconut and safflower oils. Formula feedings are particularly suitable for this type of investigation because the components can be measured accurately and all variables can be controlled.

The results obtained from the use of a formula diet containing equal amounts of coconut and safflower oils was included in our first report.[3] The results obtained from ten patients, who were placed on similar formula diets consisting of a homogenous mixture of non-fat milk solids,[¶] glucose, and safflower oil or a mixture of safflower and coconut oils, are reported herein. However, a formula containing 20 per cent fat rather than 40 per cent (used in the first portion of our study) was used, in order to determine whether or not the results of the previous study could be reproduced by simply reducing the total fat content by 50 per cent.

MATERIALS

The formula diet, originated by Ahrens et al.,[2] was used. In the first part of our study, we utilized a caloric composition of 42 per cent fat, 43 per cent carbohydrate and 15 per cent protein. This formula was modified in the second portion of our study to contain 21 per cent fat, 64 per cent carbohydrate and 15 per cent protein. One gm. of NaCl and two multivitamins were given as daily supplements. Clinical data concerning the patients used in the study

¶ Non-fat milk solids were supplied through the courtesy of the Carnation Company, Boston, Mass.

TABLE I

Clinical Data on Patients Used in the Study

Case No. (and Patient)	Age	Occupation	Height (inches)	Weight (pounds)			Clinical Diagnosis*
				Maximum Prestudy	Study	Poststudy	
1 (E. DuB.)	43	Salesman	72	185	177	174	MI
2 (J. H.)	50	Nurses aide	70	203	195	169$^{1}/_{2}$	MI
3 (J. G.)	39	Truck driver	72	190	183	178	MI
4 (A. L.)	67	Salesman	63	183	164	161	MI
5 (J. G.)	50	Gardener	65	190	155	160	MI
6 (J. A.)	48	Truck mechanic	70	235	204	†	MI
7 (W. G.)	37	Machinist	64	220	166	152	MI
8 (W. A.)	54	Salesman	66	175	164	137	AP
9 (E. G.)	40	Social worker	68	195	192	156	AP
10 (E. C.)	46	Factory worker	65	162	143	143	MI

* All patients had coronary heart disease. MI = myocardial infarction; AP = angina pectoris.
† Patient lost to follow-up.

are provided in Table I. All patients had coronary heart disease; eight showed old or stable myocardial infarction by electrocardiogram, and the remaining two had angina pectoris. Constant body weight was maintained throughout the study by adjusting the amount of formula given. Six of the patients were obese.

METHODS

Patients were referred to the Nutritional Study Unit at the Veterans Administration Hospital by the Medical Service. Samples of blood were drawn from the patients to determine the presence of hypercholesteremia. Serum cholesterol was measured by the Sperry and Webb method,[5] which makes possible definite determination of the cholesterol content by using only a single sample of blood. Subjects with diseases of the liver or kidneys or endocrine dysfunctions were omitted from this study.

Patients selected for study were placed at random into Groups A and B. Control determinations of serum cholesterol were obtained and analyzed in duplicate immediately prior to the initiation of the formula regimen. The subjects were studied during two periods of two weeks each. The members of Group A were given a formula containing 20 per cent

fat (safflower oil) during the initial two week period. Fats in non-fat milk solids were present in negligible amounts. During the second period, they were again given a 20 per cent fat formula, however, this time the fat consisted of an equal mixture of safflower and coconut oils. Subjects in Group B were fed in the reverse, receiving the safflower-coconut oil mixture in the first period and the formula with only safflower oil in the second period.

On the first day of the study, two blood samples were drawn, two hours apart, from the fasting patients. These were analyzed in duplicate for levels of cholesterol. At the end of the second week, prior to changing the formula, two similar samples were again drawn. At the end of the fourth week, another two samples were obtained. Following the study period, the obese patients were placed on weight reduction diets (Table I).

Serum cholesterol determinations were also performed on the first, seventh, fourteenth and twenty-eighth days. Samples obtained on the first, fourteenth and twenty-eighth days were drawn from the fasting patient at 8:00 A.M. and again at 10:00 A.M. Double samples were used in an effort to eliminate error due to hourly variations.

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TABLE II
Mean Cholesterol Values

Group and Subject	Mean Serum Cholesterol (mg./100 ml.)		
	Control	Safflower	Mixture
Group A			
1	327	196	227
3	333	206	249
5	411	307	348
7	380	294	336
9	385	307	328
Mean	367	262	298
Group B			
2	320	249	240
4	330	251	261
6	249	204	220
8	403	370	362
10	396	282	246
Mean	340	271	266
Grand Mean	358	264	284

RESULTS

Changes in the levels of cholesterol in the serum of ten patients, who were placed on formula diets, are indicated in Table II. Mean values of the different phases of the study are also recorded in this table.

The formula containing 20 per cent safflower oil lowered the levels of cholesterol in the serum significantly, as did the formula consisting of 20 per cent coconut and safflower oils. Levels of lipids in the blood were lowered more effectively in man by the safflower oil formula than by the mixture.

During the second two week period, the 20 per cent safflower oil formula maintained lowered levels of cholesterol in the serum, whereas the levels increased slightly when the formula with the mixture was administered (Table II).

COMMENTS

Our previously reported investigation[3] indicated that formulas containing 40 per cent fat, either safflower oil or safflower and coconut oils, were equally effective in lowering cholesterol levels in human serum. This

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second investigation indicates that a comparable lowering effect is obtained with a formula containing 20 per cent fat consisting of only safflower oil.

The current literature evidences concern with the effect of dietary fat, both the quantity and quality, on human metabolism as applied to the etiology of disease of the coronary arteries. We have demonstrated that the effect of lowering the levels of cholesterol in the serum can be accomplished just as well with a formula containing only 20 per cent fat. With the data available from this and the first part of our study, no one level of total fat or unsaturated fat can be chosen as optimal. The results of three of the four formulas were almost identical. The only formula which did not show appreciable changes was that with the 20 per cent fat mixture. Some observers[6] have stated that total fat, and others that total unsaturated fat, are the significant elements in the production and reduction of cholesterol in the blood. Our results show that wide variations in both produce similar results. Perhaps the optimal level is within the 20 to 40 per cent range of total fat and unsaturated fat. Further work with both higher and lower variations of total and unsaturated fats should be carried out.

The number of patients and the duration of study were not sufficient to enable evaluation of any effect on atherosclerotic processes.

SUMMARY

Formula type feedings, utilizing reduced quantities of safflower oil and safflower-coconut oil mixture, were administered to ten patients with hypercholesteremia. It was observed that similar reduction in levels of cholesterol in the serum can be obtained with this type of regimen as compared to that using a formula which contains twice the amount of fat in the same proportions. A slightly greater reduction of cholesterol in the serum was obtained when a formula containing 20 per cent safflower oil was used.

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Excretion of Urinary Metabolites in Calcium Oxalate Urolithiasis

Effect of Tryptophan and Vitamin B$_6$ Administration

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WITH THE TECHNICAL ASSISTANCE OF FAROUK F. FARAGALLA, GRACE S. H. SHEN AND
MARY M. KEARNEY

APPROXIMATELY two-thirds of human kidney calculi are composed of either pure calcium oxalate or calcium oxalate mixed with apatite.[1] Although considerable clinical observation and research has been devoted to this problem, little is known regarding the cause and prevention of oxalate calculi. Recent studies in this laboratory have associated increased excretion of oxalate in the urine with vitamin B$_6$ deficiency.[2,3] Renal calculi of calcium oxalate monohydrate have been produced in vitamin B$_6$ deficient rats[4] and oxalate nephrocalcinosis has been observed in vitamin B$_6$ deficient cats.[3] In both of these species, the pathologic processes observed resembled their human counterparts. These observations have supplied a new approach to the study of diseases of the kidney, associated with oxalate deposition. The present study was undertaken to compare the urinary excretion of various metabolites, including some associated with vitamin B$_6$ in normal adults and persons with histories of oxalate renal calculi. The effect of the administration of vitamin B$_6$ and tryptophan on the excretion of some of these metabolites has also been investigated.

EXPERIMENTAL

The subjects studied were twelve normal

adults and eighteen adult patients whose records evidenced recurrent formation of pure calcium oxalate kidney stones. Two twenty-four hour collections of urines or the control samples were obtained from each of these people. The patients were then fed 10 gm. of DL-tryptophan and another twenty-four hour urine specimen was collected. After a lapse of at least two more days, all of the subjects took 2 doses of 20 mg. pyridoxine HCl, orally, twenty-four hours apart and, following the second dose, another twenty-four hour urine collection was made. The samples of urine obtained during each collection period were analyzed for creatinine,[5] xanthurenic acid,[6] oxalic acid,[7] citric acid[8] and 4-pyridoxic acid.[9] The control samples were also analyzed for sodium and potassium (using the method of flame photometry), nitrogen (employing Kjeldahl's method), calcium,[10] magnesium,[11] phosphate phosphorus[12] and chloride.[13] All samples were preserved under toluene and were refrigerated or frozen. During the experimental period, the subjects ate their usual diets. None of the subjects were receiving vitamin therapy before the experimental period began.

RESULTS

The results of the analyses of the control samples of urine are provided in Table I. Patients with histories of oxalate calculi did not excrete significantly more oxalate than the normal subjects. However, they did excrete significantly more xanthurenic and pyridoxic acids and less citric acid than the normal subjects.

The citric acid excretion values of only

TABLE I

Twenty-Four Hour Excretion of Metabolites by Normal Adults and Oxalate Calculi Formers (Patients)

Metabolite	Normal Subjects* (12)	Patients* (18)	Statistical Significance†
Creatinine (gm.)	1.53 ± 0.11	1.51 ± 0.10	NS
Xanthurenic Acid (mg.)	6.8 ± 0.6	22.4 ± 5.1	p<0.01
Oxalic Acid (mg.)	37.8 ± 2.4	39.4 ± 2.7	NS
Citric Acid (mg.)	813 ± 60	535 ± 77‡	p<0.01
Pyridoxic Acid (mg.)	3.1 ± 0.3	5.0 ± 0.6	p<0.01
Calcium (mg.)	295 ± 26	377 ± 36	NS
Magnesium (mg.)	79 ± 6	72 ± 5	NS
Nitrogen (gm.)	14.8 ± 2.4	11.3 ± 0.7	NS
Phosphate phosphorus (mg.)	930 ± 49	889 ± 61	NS
Sodium (mEq.)	168 ± 10	191 ± 19	NS
Potassium (mEq.)	65 ± 4	54 ± 5	NS
Chloride (mEq.)	170 ± 15	188 ± 43	NS

* All values include the standard error of the mean.
† NS = not significant.
‡ Includes citric acid values of only twelve patients.

twelve of the patients are included in the table. Six of the patients had had kidney infections and it was felt that the low excretion of citric acid by these persons (mean of 164 mg. per 24 hours) might be, at least in part, the result of bacterial action. Values for the excretion of calcium, magnesium, nitrogen, phosphate phosphorus, sodium, potassium and chloride were not significantly different in the two groups. The nitrogen excretion values for the normal subjects included one high value obtained from a subject who ate a diet with an extremely high protein content. The mean value of the

normal group, excluding this person, was 12.4 ± .8 gm. of nitrogen per hour.

In this type of study there are always difficulties in providing adequate control. The diets of the subjects were not the same and the average age of the patients was forty-six years while that of the normal subjects was thirty-one years. However, the almost identical creatinine excretion values obtained are an indication of the similarity in muscle mass of the subjects in both groups. Creatinine values, also were of use as a check on the completeness of individual twenty-four hour urine collections.

Summarized in Table II are the changes from the control values for the urinary excretion of several metabolites after the administration of tryptophan and vitamin B_6. Following the feeding of tryptophan, a significant rise in oxalic acid excretion of the patients occurred. Only three of the normal subjects received tryptophan loads and their oxalic acid excretions increased 12.6, 16.3 and 21.7 mg., respectively. The administration of vitamin B_6 resulted in a decrease in oxalate excretion by all but one of the patients and one of the normal subjects. The latter showed a large increase in oxalic acid excretion. If the value obtained for this person had not been included in the data in Table II, the change in oxalic acid excretion of the normal subjects, following vitamin B_6 administration, would have been −5.4 mg. instead of −3.6 and the difference would have

TABLE II

Changes in Twenty-Four Hour Urinary Excretion of Metabolites Following Tryptophan and Vitamin B_6 Administration

Metabolite	Normal Subjects (10)	Patients (18)	
	Vitamin B_6	Tryptophan	Vitamin B_6
Oxalic acid (mg.)	−3.60 NS*	+9.60 p<0.01	−5.30 p<0.01
Xanthurenic acid (mg.)	−2.00 NS	+24.40 NS*	−6.20 NS
Citric acid (mg.)	−61.00 NS	−28.00 NS	+2.00 NS
Pyridoxic acid (mg.)	+5.20 p<0.001	−0.07 NS	+6.80 p<0.001
Creatinine (gm.)	−0.08 NS	+0.06 NS	+0.13 NS

NOTE: Significance determined by t test on the differences between control values and those obtained following the load tests. NS = not significant. * Lack of statistical significance due to one abnormal value in each group (see text).

been significant at less than the 0.01 level.

Changes in xanthurenic acid excretion, following the tryptophan and vitamin B_6 loads, were in the same direction as those obtained for oxalic acid. The mean increase of 24.4. mg. in the xanthurenic acid excretion of the patients was distorted by an increase of 238.8 mg. in the urine of one of the patients. Fifteen of the eighteen patients showed an increased excretion of xanthurenic acid following tryptophan ingestion; however, the extreme value of the one patient, even though it was in the same direction as all but three of the samples, increased the standard error of the mean to a point where the t test did not give a significant difference. If this value had been omitted from the determination of the mean, the change in the xanthurenic acid excretion of the patients would have been +11.8 mg. and the difference would have been significant at less than the 0.01 level. The administration of vitamin B_6 resulted in decreases in the xanthurenic acid excretion of thirteen of the eighteen patients and eight of ten of the normal subjects.

Neither the administration of tryptophan nor of vitamin B_6 had a significant effect on the excretion of citric acid by the subjects; the normal controls continued to excrete considerably more citric acid than the patients during all the collection periods.

Excretion of 4-pyridoxic acid was not affected by the tryptophan feeding and, as expected, increased after the administration of vitamin B_6. Neither of the loads affected the excretion of creatinine significantly.

<div align="center">COMMENTS</div>

Both in this study and a previous study of mentally deficient children,[14] the administration of vitamin B_6 to individuals presumed to be receiving a diet adequate in vitamin B_6 resulted in a significant decrease in oxalate excretion. Although the decrease observed in this work was not large, averaging approximately 15 per cent, it was observed in all but two of the subjects. In areas of the world where people are poorly nourished and among people subjected to nutritional stresses brought about by war, the

incidence of kidney disease, associated with oxalate deposition and oxaluria, is increased.[3] The amount of oxalate excreted by these people and the effect of vitamin B_6 on their excretion of oxalate have not yet been studied.

Presumably, the formation of urinary calculi of calcium oxalate must be related to an increased concentration of oxalate or calcium in the urine, to altered solvent characteristics of some urines toward calcium oxalate, or to both. It has not yet been determined whether or not continuous supplementation with vitamin B_6 can bring about a sustained decrease in oxalate excretion. This would be of some importance since it can be argued that the risk of formation of oxalate stones might be lessened if urinary oxalate were decreased.

Until recently, urinary oxalate was generally considered to be almost entirely exogenous in origin. It has now been shown in human beings[15] and animals[2] that much of it may be derived endogenously from glycine. In the study reported herein, the feeding of tryptophan resulted in a rise in oxalate excretion from all but three of the subjects tested. Other studies are needed to determine whether or not the tryptophan acts as an oxalate precursor, or alters the metabolism of the subjects in another way so that more oxalate is produced from other precursors.

Although vitamin B_6 is involved in many fundamental biochemical reactions, methods for the measurement of the adequacy of vitamin B_6 nutriture have not been satisfactory. Intermediates of tryptophan metabolism excreted in the urine, particularly xanthurenic acid which increases in subjects with vitamin B_6 deficiency, are often used as measures. The major metabolite of vitamin B_6 in the urine, 4-pyridoxic acid, has also been used in assessing vitamin B_6 nutriture. Significantly more xanthurenic acid was excreted in control urines from the patients than in those from the normal subjects. The most xanthurenic acid excreted by any of the normal subjects in their control samples was 10.9 mg. per 24 hours. Ten of the eighteen patients showed more than twice the mean for the excretion of xanthurenic acid of the normal subjects, and five of the ten patients excreted more than 30 mg. per 24

hours, an amount which Vilter et al.[16] consider to be abnormal even following a tryptophan load test. In the twenty-four hours after the administration of tryptophan, eleven of the eighteen patients excreted more than 30 mg. of xanthurenic acid, but, following the administration of vitamin B_6, only one person's excretion of xanthurenic acid remained at more than 30 mg. per day. The highest control value for a patient was 71.2 mg. per 24 hours; following the tryptophan load test, she excreted 310 mg. of xanthurenic acid in the twenty-four hour period. This patient was the only one who experienced gastric distress following receipt of the tryptophan load. After administration of vitamin B_6, her excretion of xanthurenic acid in twenty-four hours dropped to 4.6 mg.

Unfortunately, the method commonly used in measuring 4-pyridoxic acid in urine also measures fluorescent substances other than 4-pyridoxic acid. Thus, the values reported for 4-pyridoxic acid are probably somewhat in error. This would be particularly true if the subjects being studied had been consuming diets deficient in vitamin B_6. The people used in this investigation consumed their usual mixed diets which presumably contained 1 to 2 mg. of vitamin B_6 per day (the suggested requirement by the National Research Council).[17] The control samples from the normal subjects contained significantly less 4-pyridoxic acid than did those from the patients. As expected, in both groups there was a marked increase in the excretion of 4-pyridoxic acid following receipt of the vitamin B_6 loads. This increase was slightly greater in the urines of the patients; in the twenty-four hours following the administration of the pyridoxine loads, the normal subjects excreted 8.2 ± 0.8 mg. of 4-pyridoxic acid (p <0.01) and the patients, 11.9 ± 0.9 mg.

It is impossible at this time to properly determine the meaning of the data concerning xanthurenic and 4-pyridoxic acids obtained in this experiment. Certainly, it appears that there are significant differences within the groups in the excretion of these two metabolites associated with vitamin B_6 nutriture. The data concerning xanthurenic acid excretion are particularly interesting because, of the eighteen

patients studied, six showed control levels of xanthurenic acid that were less than the mean of the normal subjects, but nine excreted an amount of xanthurenic acid considerably more than twice the highest control value of the normal subjects. If these higher values for excretion of xanthurenic acid can be interpreted as representing a metabolic abnormality, then apparently such an abnormality is not present in all of the patients.

In this and other studies,[18] no significant difference in urinary oxalate excretion has been observed in people, with or without histories of renal oxalate calculi, in well nourished populations. These observations suggest the possibility that the solvent characteristics of urine rather than the quantity of oxalate are primarily responsible for the formation of urinary stones. Miller et al.[19] have shown that calcium oxalate is extremely soluble in urine in comparison to water. They found that citric acid in particular and various electrolytes markedly increased the solubility of oxalate.

In our study, no significant differences were observed in the urinary excretion of electrolytes. However, normal subjects excreted significantly more citric acid than the patients. A number of investigators[20-22] have pointed out that decreased excretion of citrate occurs in subjects with calcium nephrolithiasis. They have suggested that decreased citrate excretion might be of importance in the etiology of calcium calculi. Conway et al.[23] consider any gross diminution of urinary citrate to be due to infection of the urinary tract. We have not included in the tables of this report, the values for the excretion of citric acid of six of the patients with histories of urinary infections. The citric acid excretions of all of these subjects were considerably below the mean of the other patients. Harrison and Harrison[24] have found that calcium phosphate crystals precipitate in the kidneys of rats in which the urinary citrate excretion is inhibited by acetazoleamide. In our laboratory,[4] it has been demonstrated that acidification of the urine (a process which decreases citrate excretion) enhances the development of calcium oxalate concretions in the kidneys of rats with vitamin B_6 deficiency. It seems possible that the formation of oxalate

stones in the people studied could have been caused, in part, by a decreased solubility of oxalate in urine, related to the urinary citrate concentration.

SUMMARY

Studies of the urinary excretion of a number of metabolites by normal adults and by persons suffering from chronic formation of calcium oxalate calculi have been made. The normal subjects excreted significantly less xanthurenic acid and 4-pyridoxic acid and more citric acid than the patients.

Following administration of tryptophan, there was a marked rise in the excretion of oxalate. In all but two of the subjects, ingestion of vitamin B_6 was followed by a decrease in urinary oxalate.

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Experiences with Metrecal®

H. J. ROBERTS, M.D.

THE EFFECTIVE management of obesity, a disorder affecting on an average of one out of four patients in the United States, continues to be a great enigma and challenge to practicing physicians. Numerous misconceptions and other obstacles to successful reducing, pertaining to diets, drugs and exercise, are problems for both the patient and the physician. Crash programs, do-it–yourself diets, high protein diets, posthypnotic suggestion, vitamins and undue reliance on the anorexigenic agents are only a few examples. Unfortunately, superimposed upon these factors are the impracticability of diets handed to patients, the lack of enthusiasm by the physicians and insufficient professional time devoted to adequate instruction and supervision during this necessarily long, drawn-out process.

There are probably as many authoritative regimens for weight reduction as there are "authorities" and interested physicians. Every clinician who attempts to cope intelligently with this problem finds that he must personally extract from the amassed knowledge of the medical, dietetic and pharmacologic sciences, from his own observations in therapeutics and diet therapy, and from the effective methods of handling obese patients psychologically, those technics which appeal to him as being the most pertinent and practical. Wide differences of opinion exist, so that a seemingly sound and successful program practiced by some clinicians is frowned upon by others. Current attitudes concerning weight reduction are reviewed in several recent papers and books.[1-5]

MANAGEMENT

With the majority of *well motivated* patients

with obesity, I have found that a program such as the following is usually successful.

(1) The patient is properly indoctrinated in the basic elements of weight-reducing diets. Stress is placed both upon those calorie-laden foods which generally are to be avoided and upon appetite-satisfying foods that need not be curtailed. Specific attention is given to such factors as alcohol, salt, fluids and snacks, according to the patient's needs.

(2) The importance of the following is discussed: (a) correct spacing of meals; (b) a breakfast containing adequate protein in order to take advantage of its unique specific dynamic action; and (c) "scientific nibbling," particularly in the case of "night eaters."

(3) The choice of a calorie-deficient diet is based upon the estimated daily energy requirements of the patient; however, it is one which will not lead to undue negative nitrogen balance or other severe insults to the body's economy. This is particularly necessary with elderly patients, in whom excessive and rapid weight reduction is undesirable. Such hypocaloric diets for women generally contain 1,000 or 1,200 calories, and for men, 1,200 to 1,800 calories. Their simplicity and effectiveness is enhanced by utilizing the system of "exchange foods." Furthermore, in making up the diet such practical features as food likes and dislikes, recreational activities and the availability of certain foods during work must be taken into account. The keeping of a diary of the actual diet for one or several weeks prior to and following the institution of a therapeutic diet is often of great practical value for both the patient and the physician.

(4) The importance of the following is stressed: (a) being socially unobtrusive about

dieting; (b) minimizing use of the term "diet"; (c) being aware of the dangers of early overconfidence and bragging; (d) avoiding weighing oneself too frequently (once or twice a week is usually sufficient); (e) the lifelong nature of this "battle of the bulges" that is being undertaken; and (f) premeditated graceful responses to offers of cocktails, desserts or extra foods in the presence of company.

(5) The patient is ordered to follow a program of moderate activity that can be readily incorporated into his daily routine. Particular emphasis is placed upon walking whenever possible. Simple muscle-toning exercises are readily demonstrated and easily learned. Such exercises are more meaningful and more apt to prove successful than unaccustomed push ups or week end splurges of golf and tennis.

(6) The necessity of enthusiasm for one's work and recreational pursuits is stressed. Activities centering about creative hobbies, involving use of the hands, are excellent for minimizing the boredom and worry of the "white-collar" worker which so often precede food binges. Before the patient embarks upon such activities, however, issue should be taken with the old adage that "anything worth doing is worth doing well" to avoid premature discouragement of perfectionists.

(7) The value of the expressed patience, encouragement, cooperativeness and enthusiasm of the physician and others whose assistance he might seek (viz., dietitians, religious or social leaders, and marital counselors, is emphasized. Concomitantly, there should be a forthright explanation by either the physician or nutritionist that the patient's obesity is being regarded as a disease and not as a vice. In most instances, this is most effectively demonstrated by the willingness of a busy physician to give these patients adequate time at frequent intervals and for nominal fees.

(8) The physician should be wary of the belief that patients can be talked or read out of obesity by means of lectures or pamphlets dealing with increased liability to poor health incurred by overweight. It is now realized that the patient's acquisition of insight, concerning both the cause and mechanisms of

his obesity, does *not* assure his ability to control these factors.

SUBJECTS

A program such as this might not be sufficient for a significant segment of the population of obese patients. This segment consists of the following three groups.

Group I: Patients with serious and progressive medical disorders, making prompt weight reduction mandatory. These conditions include coronary (ischemic) and other forms of heart disease, diabetes mellitus, rheumatoid arthritis or osteoarthritis actively involving the weight-bearing joints, recurrent thrombophlebitis of the lower limbs, peripheral vascular insufficiency, and sciatic neuritis (either primary, or secondary to a herniated intervertebral disc).

Group II: Patients requiring surgery in the near future, whose massive panniculus poses a decidedly increased hazard in terms of technical difficulties and postoperative complications (viz., anesthesia risk, fat embolism, thromboembolism).

Group III: Patients (possibly in either of the aforementioned categories) who have become discouraged after a number of futile attempts to reduce, with or without medical supervision.

It has been my repeated observation (in a practice devoted to internal medicine) that the greatest number of medical problems in which there is a pressing need for weight reduction involve *moderate* degrees of obesity, generally ranging from 20 to 30 pounds above the patient's ideal weight.

Various methods for achieving a predictable and accelerated loss of fat have been proposed. One such plan consists of total acaloric fasting for four to nine days, followed by a diet of 600 to 800 calories consisting mainly of dextrose.[6] In addition to the standard low calorie diets devised by nutritionists, numerous and varying restricted diets have been recommended, such as the reduction of all portions from one-third to one-fourth (the "social diet"),[7] a 550 calorie diet consisting of only two meals daily for forty days,[8] and the packaging of the caloric equivalent of 1,100

calories for breakfast, lunch and dinner.[9] Unfortunately, it has been the repeated experience of enlightened nutrition clinics (characterized by outstanding patient cooperation), in which obese patients were placed under conditions as conducive as possible to maximum success in weight reduction, that reasonable success is attained in only one fourth of the patients. One fourth of the group demonstrate a slight degree of success, while the remaining half may be regarded either as complete failures or hardly worth while.[10]

The substitution of natural food by diverse nutrient mixtures or formulas has been advocated since 1866 when Karell suggested the use of a liquid diet consisting of several glasses of creamy milk daily. This particular regimen has been modified through the years. For example, Moritz recommended the use of whole milk or buttermilk in 1908. In 1934, Harrop described the successful management of obesity by alternating two week periods of a diet of skimmed milk and bananas with a conventional hypocaloric diet.[11] In 1958, Feinstein, Dole and Schwartz described their experiences with 106 clinical obese patients who were maintained on a formula of 900 calories, derived from evaporated milk, dextrose, corn oil and water, which was supplemented with a multivitamin capsule.[12]

METRECAL®

Within the past year, I have incorporated a well conceived substitute food preparation, Metrecal,* into the management of the aforementioned three groups of patients (for whom the usual weight reducing measures are not adequate).

This preparation has been well received by the majority of patients, and has immeasurably simplified for me both the initiation and maintenance of effective long term programs of weight reduction. When recommended for the type of patient just described, the advantages of this preparation include the following.

(1) It eliminates the guess work and inconvenience of calorie counting (at least in the earlier phases of the program).

(2) It gets the patient "away from the table." This obviates the initial frustration of attempting to limit the quantity of food without a lowered "appestat" (the automatic weight-regulating mechanism which operates through the appetite).[5]

(3) It completely satisfies the appetite in the vast majority of patients. This includes cooks and housewives preparing meals, and the night eaters, obese diabetic patients with voracious appetites either due to the large doses of insulin required or to partially controlled diabetes, and patients having bulimia resulting from the administration of large doses of adrenocortical steroids.

(4) It obviates the uncertainty and anxieties which many patients experience in attempting to choose foods before they have had adequate dietetic instruction. It is a frequent dietitian's observation that the patient placed on a 1,000 calorie diet, actually consumes 1,200 to 1,500 calories or more.

(5) It does not induce negative nitrogen balance caused by protein starvation. In this regard, Metrecal has been tolerated well by several patients in their seventies and eighties, with no apparent insult to the body economy.

(6) It is highly practical and stable and eliminates mixing a number of ingredients. This has proved to be a particularly valuable feature for male patients who travel considerably in the course of their work.

(7) It can be employed as either the sole source of calories and nutrition, or it may be administered in a flexible manner, alternating with a hypocaloric diet. The latter technic prevents monotony and acts as an effective transition to the completely restricted diet.

(8) It has been followed in every instance by early satiety with small quantities of food when a hypocaloric diet is introduced.

(9) It will always effect weight reduction in a cooperative obese patient, provided the same degree of activity is maintained. This leads to enhanced confidence in and enthusiasm for the subsequent long term program. In addition, the checking of the patient's weight at frequent office visits will usually point out clearly overt "cheating" to both the physician and patient.

* Kindly supplied by Mead Johnson & Company, Evansville, Indiana.

(10) It gives the patient a definitive and reasonable time period as to when successful weight reduction might be achieved in the near future. Heretofore, the realization that weight reduction with 1,200 to 1,800 calorie diets would entail six months or longer was enough to discourage many potentially successful dieters. This factor alone may account for, in some measure, the experience that even after an intensive educational program, many patients, who are enrolled in clinics for weight reduction, are unable to continue their dietary regimens over prolonged periods.[13]

(11) It gives the physician several weeks in which to instruct the patient concerning proper diet therapy and the more pertinent pitfalls and fallacies in weight reduction.

(12) It essentially frees both the physician and the patient from the crutch of the anorexigenic drugs, the metabolic stimulants and the hydrophilic colloids. In my opinion, such agents are not only unnecessary but actually highly detrimental, physically and psychologically, to patients embarking on long term weight reduction programs. I have observed many instances in which an otherwise effective regimen was destined to fail as a result of undue reliance upon these substances. Furthermore, the use of anorexigenic drugs which act as central nervous system stimulants can intensify the insomnia of patients with the so-called night-eating syndrome.[3] Patients, beginning a long term weight reducing program, should be continually reminded that (1) this is one of the most severely disciplining experiences with which a person in our society can be challenged, and (2) any distress initially encountered will tend to wane gradually as the weeks elapse. To sidetrack or to minimize this issue at any stage, especially under a pharmacologic "umbrella," will undermine the entire program.

(13) It has been used without apparent detriment in a panorama of medical disorders. I have failed to observe any serious physical or biochemical complications. The gastrointestinal side effects and the modifications of the diet in instances of diabetes mellitus and peptic ulcer are discussed in this report.

TABLE I

Contents of One Can of Metrecal (227 gm.) Containing 900 Calories

Nutrient	Amount	
Protein	70	gm.
Fat	20	gm.
CHO	110	gm.
Ash	14.5	gm.
Calcium	2.0	gm.
Phosphorus	1.8	gm.
Sodium	0.9	gm.
Potassium	3.5	gm.
Chloride	1.6	gm.
Iron	.15	mg.
Manganese	2	mg.
Copper	1.5	mg.
Zinc	5.0	mg.
Iodine	150.0	mcg.
Vitamin A	5,000	U.
Vitamin D	400	U.
Vitamin E	10	U.
Thiamine	2.0	mg.
Riboflavin	3.0	mg.
Ascorbic acid	100.0	mg.
Niacinamide	15.0	mg.
Pyridoxine HCl	2.0	mg.
Vitamin B_{12}	2.0	mcg.
Calcium pantothenate	10.0	mg.

(14) It can induce striking decreases in the levels of cholesterol in the serum of many hypercholesterolemic obese patients—an effect shared by other effective low calorie diets. (The results of the studies concerning cholesterol are reported herein.)

(15) It does not appreciably alter the level of hypoprothrombinemia nor result in "anticoagulant escape" in patients being maintained on long term anticoagulant therapy to whom Metrecal was administered.

(16) It provides a definitive and practical method of helping patients to successfully reduce during periods of emotional crisis, a time when they would be most prone to go on food binges. Such binges are highly detrimental not only because of the weight that is regained, but also because these patients will promptly reset their diminished appestats to a much higher level. (The appestat mechanism may be likened to a conditioned reflex, and, as is the case with any conditioned reflex that has not been called forth for a period of time, it can be readily "reinforced." A minimal period of from four to six weeks is

necessary to subdue the appestat in most instances.[5])   Nothing is more demoralizing to the obese patient, who has conscientiously applied himself over several weeks or months, than is the prompt recharge of his appetite by dietary abuse during such periods of stress.

THE PRODUCT

Metrecal represents a blend of food materials providing 900 calories per $^1/_2$ pound (227 gm.) of the preparation.   The nutrients provided by this amount representing the contents of one can, are provided in Table I.

The protein content in this formula will satisfy the amount usually recommended in weight reduction programs, namely, one gm. of protein daily for each kg. of *ideal* body weight.   It is also apparent that the custom of having the patient take a preparation of vitamins and minerals to supplement his weight reduction diet is not necessary with Metrecal in most instances.

The caloric distribution of Metrecal is as follows: Protein, 30.5 per cent; Fat, 19.5 per cent; Carbohydrate, 50.0 per cent.

Metrecal contains non-fat milk solids, soya flour, whole milk powder, sucrose, starch, corn oil, coconut oil, yeast, vanilla flavor, vitamins and minerals.   The fat comprises a blend of corn oil, soya oil, butterfat and coconut oil.   The fat in Metrecal amounts to 6.3 gm. of total saturated fatty acids and 13.7 gm. of total unsaturated fatty acids per $^1/_2$ pound.

Metrecal is prepared in various manners according to individual convenience and preference. A supply for one day may be prepared by mixing the contents of the $^1/_2$ pound can with 1 quart of water, placing the powder on the surface of the water in a mixing bowl and thoroughly stirring it to a creamy smoothness with a mechanical blender, egg beater or fork. This will provide at least four large glasses of the product, each containing 225 calories. Metrecal also may be prepared in individual glass servings. This is done by measuring a half a cup of the preparation and placing it into a large glass.

Both unflavored and preflavored Metrecal were employed. The majority of my patients have accepted the vanilla-like flavoring of the preparation itself and many actually enjoyed its taste. In addition to the trial packets of flavoring supplied in the early phases of this study and the subsequent preflavored preparations,

other means of enhancing palatability have been utilized, such as combining one-half teaspoon of a non-sugar sweetening agent with one-half to two teaspoonfuls of various flavoring extracts. Some patients have preferred the addition of nutmeg, tea or ice chips, or have poured the preparation over ice cubes. Several have found that the blending of one small banana (entailing an additional 100 calories) with Metrecal not only enhances its taste but also serves to minimize any unpleasant aftertaste. The latter recently has been minimized by slight modification of the yeast employed in the earlier preparation. Another promising method of administering this substance in the interim between prescribed servings or to replace one or more of the liquid servings is in the form of flavored Metrecal wafers, one of which contains 30 calories.

METHODS

No specific and uniform plan was adopted owing to the anticipated variations necessarily stemming from the patient's underlying medical disorder, the initial weight, the actual weight loss, the tolerance for the preparation and other individual factors.   In general, there were four phases involved in ultimately achieving either the ideal weight or a reasonable goal decided upon at the onset of the program.

Phase I: Metrecal Alone

Metrecal was taken as the sole source of calories for periods of five days to five weeks. While the taking of four or five glassfuls at equally spaced intervals is generally desired, the individual patient was allowed to decide how often and how much of the day's supply he preferred to take at any one time.   The only implicit recommendation was that at least one glass be taken in the morning in order to obtain the necessary specific dynamic action of its protein and other nutrients.

Women were advised to drink a glass of Metrecal before preparing the evening meal for the family in order to minimize the temptation of sampling the food.   In the case of patients working away from home, the mixture was easily kept cool and transported in a thermos bottle.   Diabetic patients taking insulin were advised to drink a divided serving of Metrecal at 3 P.M. and at bedtime.   These patients were reminded that they could use the various non-caloric sweetening agents if desired.   Patients with active or recently active peptic

ulcers were told to distribute their daily allocation among at least six servings and add the prescribed anticholinergic and antacid agents.

Since a concomitant constipation during the first several weeks is inherent in every weight reduction program based on reduced caloric intake, the intake of additional fluids was insisted upon in the form of water, tea, coffee and clear bouillon. When the patient was known to have a long-standing functional gastrointestinal disorder, he was counseled concerning the taking of a mild antispasmodic agent several times daily and milk of magnesia or some other mild laxative, if needed.

Phase II: Metrecal Alternating with Diet

The patient entered this phase of the program depending upon the amount of weight lost, the occurrence of side effects, and other factors. Some patients actually enjoyed the preparation enough to express the desire to remain on Metrecal for one or several weeks longer. While several patients were able to go directly from Phase I (Metrecal alone) to Phase III (diet alone), this period of dietary transition proved to be a highly satisfactory technic. By this time, the patient had been indoctrined in diet therapy, and as a rule was eager and confident in his ability to carry out the diet correctly.

In the majority of instances, the patient began this program with a 1,000 calorie diet for three or four days, alternating with Metrecal for a similar period of time. One of the more convenient and effective dietary forms I employed during the first period was Form PS-1910 (Eli Lilly and Company) for diabetics. Incorporated on one sheet are the food exchange system, the distribution of such exchanges for the various meals, and sample menus. Suggestions for liquid diets to replace any of these meals are also included.

In some instances, the patient found it more convenient to carry out Phase II by combining Metrecal with food, ingesting a total of 1,000 calories daily. This was best apportioned as follows: *Breakfast*, Metrecal (1 glass), tea or coffee as desired; *Lunch*, lettuce and tomato salad as desired, clear bouillon as desired, asparagus or broccoli or greens (1 cup), meat or poultry (2 oz.), bread ($^1/_2$ slice), skimmed milk or buttermilk ($^1/_2$ cup), or tea or coffee as desired; *Dinner*, comparable to lunch, except for adding one fruit exchange, adding one vegetable such as peas, beets, carrots or onions ($^1/_2$ cup) and substituting coffee or tea for the milk beverage; *Late evening*, Metrecal. I have found this technic to be less effective psychologically and more conducive to caloric error than three to four day periods of Metrecal alone, alternating with diet alone.

Patients continued on the alternating program for as long as sixteen weeks. By this time, many patients had already approximated their ideal weight or had lost impressive amounts of fat. The precipitous gain in weight which was noted by Feinstein and his colleagues[12] when patients resumed to eat natural food after having been on a 900 calorie liquid diet (attributed to shifts in the extracellular fluid) was not evident in this series.

Phase III: Diet Alone

By the end of the second month, the majority of patients had been shifted either to normocaloric diets or to hypocaloric diets (in the case of those who needed further weight reduction). Such timing fits in well with the observations by Jolliffe that a minimal period of four to six weeks for an "appestat training" program is required to effectively dampen this mechanism.[5] The number of allotted calories in the hypocaloric diets ranged from 1,000 to 1,800, although an attempt was made to achieve a daily calorie deficit ranging from 500 to 1,000 calories, not exceeding the latter quantity. Patients were again advised that weight reduction was slower (although more predictable) at this stage and that they were not expected to lose more than $1^1/_2$ to 2 pounds of weight per week.

Several patients were allowed to change directly from Metrecal alone (Phase I) to diet alone (Phase III). This group consisted of (1) patients with long-standing obesity who lost considerable weight after several weeks of Metrecal, (2) patients whose obesity had developed precipitously over a relatively short period (particularly when taking adrenocortical steroids) and whose excess weight was

largely eliminated by Metrecal and (3) patients who preferred to eliminate Phase II.

In working with those patients who actually enjoyed Metrecal as a beverage, it was a simple matter to progressively increase their caloric intake to any specified quantity merely by supplementing the basic four glasses of this substance and the foods which had been allowed ad libitum (tea, coffee, clear bouillon and uncooked List 1 vegetables) with specified amounts of low calorie foods. This was best carried out by recommending an additional number of calories and then allowing the patient to decide how and when he would prefer to convert these into specific foods by means of the aforementioned table of food exchanges. If this program can be followed, the patient is assured not only of convenience and effective weight control, but also of adequate nutrition and satiety.

Having finally arrived at the desired weight on a hypocaloric diet, the patient was advised to continue his basic diet, supplemented by an additional 200 calories for the subsequent two weeks. If at that time no gain in weight had become evident, an additional 100 or 200 calories were incorporated into the diet and his weight was carefully observed. At this stage, or with the addition of 100 or 200 calories at a later time, most patients achieved the diet which, in calories, equaled their energy requirements. The other important aspects of a long term and effective program for maintaining a particular weight were continually re-emphasized (*vide supra*).

Phase IV: Crisis Periods. As previously noted, in any weight reduction program the physician must be cognizant of the tendency for obese patients, even those whose personalities are reasonably stable, to go on food binges during periods of emotional stress. Under these circumstances, my patients were instructed to revert promptly back to Phase I of this program in order to satisfy their appetite and to avoid undoing their hard-earned achievement. Other supporting measures, including sedation, were also employed when necessary. The patients were further advised to revert back to Phase I, as a compensatory measure, following a "food fling" on vacations, picnics or when entertaining others.

CLINICAL MATERIAL

All the patients included in this study were personally attended in my private practice of internal medicine. Study of the three groups was conducted solely on an outpatient basis. The vast majority of patients were being treated primarily for other serious medical disorders which were clearly aggravated by obesity.

The very nature of this patient-physician relationship and the concomitant attempt at maintaining an enthusiastic attitude towards the entire program resulted in a high degree of patient cooperation. No patient was started on Metrecal who was regarded as either psychologically unprepared for the disciplining experience of rigorous weight reduction or who expressed reluctance at being a "guinea pig." While the elements of fear and coercion were de-emphasized as much as possible, a sobering discussion of the reasons for the necessity of weight reduction always preceded the initiation of this program. Particular attention was drawn to the point that many patients would do well not to lose weight at all rather than to cyclically lose large amunts of weight, only then to regain it in a short period of time (primarily to prevent the acceleration of atheromatous deposits and gallstone formation). Such an understanding between the patient and physician (with occasional reference to this basic orientation once the patient was solely on a hypocaloric diet) has proved to be a valuable technic for making these patients "toe the mark."

In this study, there was virtually no recourse to appetite depressant drugs of any type. One patient with true hypothyroidism was maintained on her previous dosage of thyroid extract. Except for two patients with heart failure, diuretic therapy was not given to the patients comprising this group once they began taking Metrecal. However, in several cardiac and hypertensive patients who had been receiving therapeutic amounts of chlorothiazide for several months, the drug was administered as usual. In several instances, such medication was subsequently discontinued once the weight

TABLE II

Summary of Data for Twenty-Five Representative Patients of the Fifty-Seven Studied

Patient (No.)	Diagnosis	Sex	Age	Height	Body Build*	Initial Weight	Ideal Weight†	Metrecal Alone		Metrecal Alternating With Diet	
								Lowest Weight	Days Required	Lowest Weight	Days Required‡
1	Rheumatoid arthritis, generalized / Angina pectoris / Xanthomatosis / Probable gout	M	48	5'7"	M	180¼	146	174	7	149	122
2	Angina pectoris / Hypothyroidism, treated	F	69	5'1"	M	165	118	156	7	143	56
3	Angina pectoris / Subacute hepatitis	F	57	5'3"	M	163¼	124	157½	12	147¾	59
4	Rheumatoid arthritis of knees, active / Severe lymphedema of lower limbs	F	45	5'3"	M	154½	124	150½	4	139	39
5	Left hemiplegia	M	69	5'11"	L	199	173	192	17	185	30
6	Severe hypertension / Coronary insufficiency / Ventral hernia, massive / Chronic basilar artery syndrome	M	59	6'1¼"	L	212	187	201	7	198¼	14
7	Coronary insufficiency	F	52	5'5"	M	176	131	172	7	165	35
8	Coronary insufficiency	F	56	5'6"	L	173½	144	167	7	164¾	14
9	Diabetes mellitus with retinopathy	F	63	5'2"	M	155	121	151	13	147½	14
10	Strong family history of heart disease	F	61	5'5"	L	194	139	187½	14	182	35
11	Essential hypertension	M	48	6'0"	L	224	176	210	18	202	35
12	Angina pectoris	F	32	5'4"	L	192½	137	182¼	7	163	60§
13	Rheumatoid arthritis, active of ankles and wrists / Diabetes Mellitus	F	52	5'2"	M	134½	121	129½	17	—	—
14	Gout / Chronic postphlebitic syndrome of legs with lymphedema	M	62	6'2"	L	238	187	216	21	—	—
15	Right sciatica, active / Family history diabetes (both parents)	M	34	6'4"	L	256	200	237	21	209	105
16	Chronic hepatitis	M	72	5'7½"	M	189¾	151	182½	7	170	43
17	Angina pectoris	M	47	6'0"	L	199¼	176	190	7	181½	42
18	Hypertension / Thrombophlebitis of lower limb / Recent massive gastrointestinal hemorrhage from duodenal ulcer	M	51	5'8½"	L	212	164	203	7	198	21
19	Polyneuritis and reflex sympathetic dystrophy of lower limbs, severe / Periarthritis, both knees / Nontropical sprue	F	62	5'3"	M	173¾	124	170	9	167½	14
20	Hypertensive heart disease / Early congestive failure (hemoptysis, edema) / Osteoarthritis right knee	M	53	5'11½"	L	247	177	234¼	13	200½	91
21	Osteoarthritis / Sciatic neuritis	F	76	5'4"	L	190	137	185	14	177½	34
22	Angina pectoris	M	26	6'3"	I.	230	192	223½	7	210½	30
23	Osteoarthritis of right knee / Previous thrombophlebitis / Angina pectoris	F	67	5'2"	L	213½	130	207¼	7	200	30
24	Angina pectoris	M	37	6'0"	L	227½	177	217¾	14	213	14
25	Right sciatica / Osteoarthritis	F	79	5'4"	L	190	137	185½	14	180½	14

* Small frame = S; Medium frame = M; Large frame = L.
† Interpolated "ideal" weight for height, sex and body build according to tables compiled by the Metropolitan Life Insurance Company. Deviations average 5 pounds for a medium frame and 7 pounds for a large frame.
‡ Excluding Phase I (Metrecal alone).
§ Not consecutive.

NOTE: Observations of individual patients (patient no. indicated in parentheses) are as follows.

(1) Appetite suppressed in spite of being maintained on prednisone; no flareup of underlying gout.

(2) Gratifying improvement of angina pectoris and neuritic pains in lower limbs; appetite suppressed completely on Metrecal even though taking 1 gr. thyroid daily; cholesterol decreased from 311 mg. per cent to 223 mg. per cent after 4 weeks.

(3) Although required to do a great deal of cooking, she found that Metrecal prior to preparing meals allayed her appetite; subsequently continued to lose to 142½ pounds on diet alone, taking Metrecal for several days as appetite began to increase.

(4) Gratifying relief of both lymphedema (without diuretics) and of pains in the knee; patient received dexamethasone during this time without increase in appetite.

(5) Hemiplegia and sedentary existence for over one year; loss of weight helpful in subsequent rehabilitation of lower limbs; cholesterol decrease from 284 mg. per cent to 256 mg. per cent in 4 weeks.

(6) Blood pressure decreased after the first 7 days from 230/140 to 140/112 mm. Hg; no other change in medication; in less than 4 weeks, blood pressure was 140/94 mm. Hg—the lowest reading recorded in over two years of continual observation; gratifying concomitant relief of both angina and dizziness; subsequent loss to 193½ pounds on diet alone.

(7) Prothrombin time unchanged in three determinations; cholesterol decreased consecutively from 280 mg. per cent to 200 mg. per cent at the end of 6 weeks; further weight loss to 162 pounds in 1 month on diet alone.

(8) No change in prothrombin time (two determinations); one glass of Metrecal prior to making family evening meal prevented nibbling.

(9) Voracious appetite due to preceding incompletely controlled diabetes and 45 U. of NPH insulin daily, resulting in an 11½ pound weight gain; appetite completely satisfied by Metrecal; no insulin reactions with proper spacing of the Metrecal, particularly at 3 P.M. and at bedtime.

(10) Cholesterol decreased from 240 mg. per cent to 219 mg. per cent.

TABLE II (*Continued*)

(11) Gratifying reduction in long-standing hypertension (averaging 200/120 mm. Hg) to normal levels (averaging 140/86 mm. Hg) with loss of associated symptoms; also successful in concomitantly cutting down on his excessive smoking of cigars; reduction in serum cholesterol from 283 mg. per cent to 175 mg. per cent in 4 weeks.

(12) Prompt amelioration of anginal pains; continued on Metrecal alternating with diet for another 10 days when she experienced severe coronary insufficiency after excessive exertion; hospitalized because of recurring anginal pains; Metrecal resumed after hospitalization with loss of both pain and weight.

(13) Required increasing amounts of dexamethasone for active rheumatoid arthritis; voracious increase in appetite ensued resulting in a weight gain of $9^3/_4$ pounds within 1 month; Metrecal completely suppressed appetite during weight reduction while on the same dose of dexamethasone.

(14) Marked relief of lymphedema; no flareup of previously recurrent gout.

(15) Gain of 7 pounds on vacation during Phase II was again lost after resuming Metrecal alone for 10 days; marked improvement in sciatic neuritis.

(16) Striking improvement in appetite and strength; previous bromsulphalein excretion (11-12-59) of 28.5 per cent retention at 45 minutes was reduced to 15.7 per cent (12-10-59).

(17) Prompt subsidence of angina pectoris; a habitual cigar smoker, but was able both to completely stop smoking and to lose weight during and after Metrecal with little difficulty.

(18) Blood pressure decreased from a previously consistent level of 210/120 to 120/90 mm. Hg, all previous antihypertensive medication having been discontinued; complete healing of active peptic ulcer after recent massive gastrointestinal hemorrhage (requiring 10 pt. of blood several weeks previously in the hospital); Metrecal given at frequent intervals, alternating with an antacid preparation and an anticholinergic drug; patient also placed on Metrecal in view of the possibility of subsequent gastric surgery; successfully continued to lose weight at an average of 2 pounds weekly when placed on a hypocaloric diet alone.

(19) Tolerated Metrecal well; previous sprue had been actively treated with vitamin B_{12}, folic acid and a wheat free diet; patient completely confined to bed and a wheel chair owing to severity of her long-standing reflex sympathetic dystrophy, neuropathy and arthropathy, but still was able to lose weight for the first time in over five years.

(22) Patient promptly lost all evidence of heart failure when weight was reduced through employment of Metrecal alone; required no further orthopedic attention for previous difficulty with right knee; cholesterol decreased from 243 mg. per cent to 188 mg. per cent in 5 weeks.

(21) Successful weight loss in spite of obesity for over four decades; continued to lose weight on hypocaloric diet alone.

(22) Prompt cessation of anginal pains after first week of weight reduction.

(23) Blood pressure decreased from 210/110 to 120/82 mm. Hg within 2 weeks of weight reduction; although undoubtedly cheating, still was able to lose weight for the first time in a number of years; also marked improvement of right knee, previously requiring frequent injections of intra-articular hydrocortisone.

(24) Loss of anginal pains after third week of weight reduction.

(25) In spite of her age and the duration of obesity (four decades), this patient promptly lost weight without any apparent harmful effects.

reduction *per se* profoundly lowered the blood pressure or caused the edema to regress and the myocardial efficiency to increase.

It is of some interest that male patients represented approximately 45 per cent of this group. This is in contrast to the 15 per cent reported by large clinics for obesity.[10,14] I was repeatedly impressed by the appreciation of these male patients for the twofold convenience of Metrecal in terms of losing weight and at the same time allowing them to pursue their occupations with little dietary inconvenience in the form of complex menus and calorie computations. This appreciation and enthusiasm was noted particularly in patients having angina pectoris.

RESULTS

Of the fifty-seven patients whose weight reduction programs were initiated with Metrecal, seven dropped out of their own accord. Three of these developed diarrhea, three believed they could not tolerate the cramps from constipation or other gastrointestinal disorders (one of whom had disturbing psychologic manifestations) while one highly nervous woman objected to the odor of the preparation. These side effects are discussed at length in this report.

After several weeks of gratifying weight loss, amounting to 8 and 10 pounds, respectively, two patients required hospitalization for medical emergencies (acute myocardial infarction). These patients were maintained on hypocaloric hospital diets in order to avoid undue conspicuousness and questioning on the part of the nursing staff and other patients (since Metrecal had not yet been released). These patients were not regarded as treatment failures. One patient (no. 12 in Table II) continued to lose weight on Metrecal when it was resumed following hospitalization.

In this study, I considered the *index of failure* of the program to be the patient's inability to lose at least 5 pounds within the first month of treatment. (This is in contrast to 5 pounds in not less than four months of therapy, the index of failure employed by Trulson, Walsh, and Caso.[14]) Five of the patients (9 per cent), who were participating in Phases I and II for at least four weeks, were regarded as treatment failures. However, the majority of these failures actually showed significant weight loss after being on a restricted diet during the ensuing three to six months.

The total rate of failure for Phases I and II (including those who dropped out of the study) was, therefore, 21 per cent or 12 patients. In Feinstein's study 35 per cent of 106 patients discontinued the 900 calorie formula diet.[12]

Weight Loss

The average total weight loss during the first week of Metrecal therapy was 6.3 pounds (ranging from 1 to 12 pounds). By the end of the first month of therapy, the average total
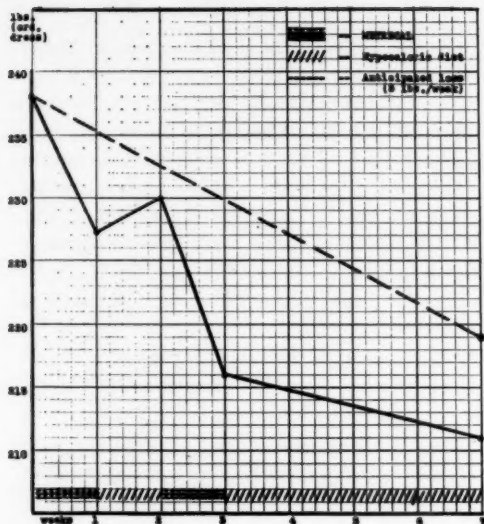
FIG. 1. Weight reduction graph of patient No. 14 (see Table II).

FIG. 2. Weight reduction graph of patient No. 20 (see Table II).

weight loss was 12.8 pounds (ranging from 4 to 24 pounds). This includes those patients, who were solely maintained on Metrecal, and the larger group in Phase II, who by this time were alternating Metrecal with the 1,000 calorie diet. The average total weight loss at six weeks on either Metrecal (alone or alternating with a hypocaloric diet) or diet alone (Phase III) was 16 pounds (ranging from 8 to 25 pounds).

Table II summarizes the more pertinent data pertaining to actual weight reduction during Phase I (Metrecal alone) and Phase II (Metrecal alternating with diet) in twenty-five patients who are representative of the group of fifty-seven patients.

These observations are in accord with the general experience that weight loss through severe restriction of calories tends to be most profound and rapid during the first few weeks, and then proceeds at a slower steady rate. The majority of patients in this series, including several inactive cardiac and hemiplegic patients, continued to lose weight on the prescribed regimen at the rate of 1 to 3 pounds per week until their weight goal had been achieved.

Although striking amounts of weight loss were frequently evident in the markedly obese subjects, no predictable correlation could be made between the degree of initial obesity and the weight loss during the early stages. Even when a fairly accurate estimation of the daily energy expenditure of the patient in terms of calories and the calorie deficit afforded by a hypocaloric diet is made, one cannot expect patients to conform uniformly to the line representing the anticipated weight loss in weight reduction graphs. For example, if the daily deficit is 1,000 calories, one might expect a 7,000 calorie deficit in seven days to become manifest as a 2 pound weight loss. (It is known that 1 pound of body fat is the metabolic equivalent of approximately 3,500 calories. Another method that can be used to predict the weekly weight loss is the multiplication of the daily calorie deficit by 0.002.)

The following additional factors must be kept in mind: (1) shifts of body water (two glasses of water can increase the weight by 1 pound), (2) variations in weight due to a full urinary bladder (the urine may weigh as much as 1 pound) or a full rectum (a normal bowel movement may weigh up to a half a pound) and (3) the reduced total metabolism with lessened body weight (there being a reduction

TABLE III
Comparison of Weight Loss with Various Regimens*

Authors	Regimen Employed	No. of Patients	Percentage who lost		
			Less Than 10 Pounds	10 to 19 Pounds	20 Pounds or More
Gray and Kaltenbach[15]	900 calorie food diet	314	52	20	28
Osserman and Dolger[16]	1,000 calorie food plus Dexedrine® (diabetic patients)	55	35	36	29
Munves[17]	1,200 to 1,800 calorie diet (groups and individual interviews)	48	71	21	8
Young et al.[10]	Varied diets (nutrition clinic)	131	40	32	28
Jolliffe and Alpert[18]	1,000 to 1,400 calories; citrus juice before meals	73	53	36	11
Feinstein et al.[12]	900 calorie formula diet	106	17	24	59
Roberts	(a) Metrecal (Phases I and II only)	57	40	47	13
	(b) Metrecal and subsequent hypocaloric diet	...	31	48	21

* This table was adapted after that of Feinstein, Dole and Schwartz.[12]

in the calorie expenditure by approximately 4 calories for each pound of fat lost).

The effectiveness of Metrecal alone and Metrecal alternating with a 1,000 calorie diet can be readily appreciated in weight reduction graphs (Figs. 1 and 2). However, the aforementioned reservations cited should be borne in mind. The estimated weight reduction is calculated at an anticipated rate of continuing weight loss, ranging from 2 to 3 pounds per week.

COMMENTS

A statistical and percentage comparison between the success of the program described herein and other weight reduction programs is influenced by the following factors: (1) the obese patients in this study did not present themselves primarily for treatment of their obesity, but rather for serious underlying organic diseases; (2) in most instances, the degree of obesity was not regarded as severe; (3) a hypocaloric diet, with or without Metrecal, was instituted in from two to six weeks, rather than conforming to a fixed and clinically undesirable program of continuing the formula alone for four to six weeks; (4) the selection of patients for this study was limited to the highly motivated clientele of a privately practicing physician; and (5) at no phase did

the patients resort to the services of a dietitian or to inpatient supervision, even though several patients could have afforded these services. The latter policy was purposefully pursued in an attempt to simulate as closely as possible the methods available to most physicians in private practice.

I am fully aware of the potentially erroneous inferences that are liable to stem from the overoptimistic results of the first two or three months of any weight reducing program. Perhaps the most valid comparison, both of the combined results of Phases I and II in this study alone and as part of a total weight reduction program (Phases I, II and III), is in the percentage of patients losing a specified amount of weight with corresponding figures from six independent studies.[10,12,15-18] The results of such a comparison, without reference to specific periods of time, are set forth in Table III. They clearly point out the effectiveness of the program employed in this study.

The success of this program may be slightly affected by the fact that few patients were obese since childhood which, admittedly, is a more difficult problem. On the other hand, my experience coincides with that of Young et al.[10] in that (1) the actual duration of obesity does not appear to be a determining factor in the

success of a weight reduction program, (2) the greatest success is achieved during the early stages (active phase) of obesity and (3) a certain degree of emotional stability and maturity is generally necessary for successful long term weight reduction and its maintenance. However, even in relatively stable patients, the ability to prevent or to minimize precipitous gains in weight during periods of emotional stress by a premeditated program such as that utilized in this study may well decide the ultimate success of a weight reduction program.

BIOCHEMICAL OBSERVATIONS

Observations on the Serum Cholesterol

The levels of cholesterol in the serum were determined, using serum from fasting patients, in the same laboratory by the same technician. Sample specimens were cross-checked with other laboratories, resulting in approximations which could be considered within the range of laboratory error. Levels above 250 mg. per 100 ml. were regarded as probably abnormal, while those consistently above 280 mg. per 100 ml. were regarded as definitely elevated.[19] Many of these patients were advised to abstain from eggs, meat fat, dairy fat and other sources of saturated fatty acids for varying periods of time prior to the initial chemical determinations. Furthermore, several had received cholesterol-lowering agents before they solicited my services.

Six of the latter patients had cholesterol levels above 280 mg. per 100 ml. (averaging 298 mg. per 100 ml.), the levels ranging from 280 mg. per 100 ml. to 341 mg. per 100 ml. After six weeks of Metrecal and a hypocaloric diet, the average level of cholesterol in the serum of these patients was 220 mg. per 100 ml. (ranging from 175 mg. per 100 ml. to 259 mg. per 100 ml.). In the remaining patients with initial cholesterol levels of less than 280 mg. per cent (averaging 231 mg. per 100 ml.), the average cholesterol level after six weeks of the described program was 205 mg. per cent. It is apparent that these quantitative changes are subject to some reservation in light of the limited number of patients studied.

The decreases observed in the group of hypercholesterolemic patients were not unexpected in view of both the limited caloric content and the composition of Metrecal. Recall that the caloric distribution from the fat in Metrecal is only 19.5 per cent (20 gm. fat per $^1/_2$ pound) which consists of approximately 70 per cent unsaturated fatty acids. These observations concur with those of other researchers, who have carried out studies on Metrecal for six weeks or longer; namely, that hypercholesterolemic patients tend to manifest a significant lowering of the levels of cholesterol in the serum within several weeks, while persons with normal initial levels of cholesterol in the serum tend to exhibit a less striking change.[20] It is conceded that similar decreases can be produced by any effective low calorie diet.

Observations on the Prothrombin Time

I was particularly interested in observing any significant change in the level of the prothrombin time in a number of obese patients with coronary heart disease or thromboembolic disorders who had been maintained on long term anticoagulant therapy. The possibility of anticoagulant escape, either in the form of clinical bleeding or prolongation of the prothrombin time, was sought out both by means of repeated clinical visits and by frequent determinations of the prothrombin time during the first three months of the described weight reduction program. This was not observed in any patient.

The standard methods of determining the prothrombin time (employing Simplastin®) were carried out, using blood from fasting patients, by the same technician in the same laboratory. The majority of my patients can be maintained effectively and safely on an outpatient basis with prothrombin times generally not exceeding twenty-five seconds. The average prothrombin time prior to Metrecal therapy in nine patients was twenty-four seconds. The average prothrombin time after one week of Metrecal therapy was 22.4 seconds. The average prothrombin times after two weeks and six weeks of Metrecal and a hypocaloric diet were virtually the same.

SIDE EFFECTS

Significant fatigue or hypoglycemic episodes did not occur during the initial phases of this program as was feared. This observation applies both to active and relatively sedentary patients.

The monotony of Metrecal infrequently became a factor during the first five days of its ingestion as the sole nutrient. In such instances, this was obviated by the flavoring instructions, the technic of alternating Metrecal with a hypocaloric diet in Phase II (once significant weight reduction had been achieved) and by stressing the desirability of taking additional clear bouillon, tea, coffee, and in Phase II lettuce, tomatoes, cucumbers and celery (without calorie-laden salad dressings).

The tendency to become constipated was commented on by approximately a third of the patients. As a result of warning the patients initially that they might become constipated and that with both the passing of several more weeks and the drinking of acaloric fluids, their bowel patterns would return to normal, most of them minimized this effect. Constipation was further obviated in Phase II of the program when stress was placed upon ingestion of the suggested raw vegetables which contained a high content of fiber and water.

One patient clearly demonstrated the induced constipation was only minor. A forty-five year old woman with progressive angina pectoris, weighing approximately 34 pounds above her ideal weight, had had gastrointestinal symptoms for many years. On several occasions she was studied for possible gall bladder disease, hiatal hernia and other upper gastrointestinal diseases; the results were negative. Following the initial seven days of Metrecal therapy, she lost six pounds (from 175 to 169 pounds) without incident. Three days later, she discontinued taking Metrecal, supposedly because of induced constipation, nausea and gas, even though she had been taking a mild anticholinergic agent continually. Over the next two months, her weight progressively rose and the angina became more severe in spite of anticoagulant therapy, coronary vasodilators, a monamine oxidase inhibitor and other measures. At this point,

she was again placed on Metrecal alone and was advised to take occasional doses of milk of magnesia as she desired. A gratifying weight reduction occurred during the next six weeks with sufficient amelioration of the angina condition to enable her to return to work.

The problem of diarrhea occurred much less frequently; however, it actually posed more of a problem in the five patients who had it. Three of these patients discontinued taking Metrecal because of this side effect.

Other gastrointestinal symptoms relating to abdominal cramps, gas and heartburn, were encountered in ten patients. The majority of these patients were known to have long-standing functional gastrointestinal disorders. As just indicated, mild antispasmodic antacid medication effectively controlled such complaints in most instances.

No striking menstrual irregularities were described by the women who were still having periods. Several patients observed a lessening of their menstrual problems as their reduction of weight became marked. This observation has also been made by others.[21]

Several patients described either a bad taste in the mouth or a coating of the tongue after taking Metrecal. This was minimized by further diluting the preparation or flavoring as described, and subsequently changing to the more recent preparation in which the yeast was modified.

Close attention was paid to any significant psychiatric aberrations. One woman with angina pectoris, functional hypoglycemia, a diabetic glucose tolerance curve and pressing family problems became so apprehensive and anxious after three days, purportedly because of her constipation, that it was deemed advisable to discontinue the Metrecal. Another markedly obese hypertensive woman with a possible right adrenal pheochromocytoma was highly agitated by the "odor" of Metrecal after the first day and therefore discontinued taking it.

The possibility of inducing ammonia intoxication was entertained in treating to several obese patients with advanced cirrhosis of the liver, owing to the moderate amount of protein in Metrecal and its concentrated nature.

One of these patients, who was approximately 50 pounds above his ideal weight, had a porta-caval shunt that was still functioning. This patient was hospitalized for clinical ammonia intoxication approximately six weeks prior to taking the preparation, following the ingestion of a single tablet of Sparine®. His initial level of ammonia in the blood was 0.8 mg. per 100 ml. (the normal values ranging up to 0.2 mg. per 100 ml.). Following two days of Metrecal as the sole nutrient, the level of ammonia in the blood was 0.5 mg. per 100 ml.

The possibility of inducing an attack of gout also was considered. One patient with recurrent clinical gout (over a period of many years) was placed on Metrecal because he exceeded his ideal weight by 45 pounds. He lost 35 pounds in four months, but experienced no recurrent episode of gout while taking Benemid®.

One patient experienced a minor maculo-papular eruption with pruritus during two trials of Metrecal. This was controlled readily by means of an antihistamine. It is possible that the rash represented an allergic response either to the milk protein or to some other ingredient. One patient with steatorrhea had no difficulty with Metrecal.

PRECAUTIONS

There are other complications inherent in any weight reduction program that can be avoided if care is taken to observe the following. (1) Such a valuable and practical type of program should not be used as a short cut to weight reduction without a concomitant attempt to educate the patient. This must be done within the frame of reference of a long term program, with emphasis being placed on diet, exercise and an analysis of the more pertinent personality, environmental and psychiatric aspects unique to that particular patient. (2) It should never be set forth as a guaranteed method or as a panacea. (3) Only well motivated patients, who sincerely wish to lose weight, should be subjected to this program. Conversely, persons, who are being coerced into losing weight against their will by family or friends, might be harmed both psychologically and physically if such a program is "shoved down their throat," leading to wide cyclical alternations between weight loss and rebellious weight gain. (4) The physician must be sure that the patient is actually in need of vigorous weight reduction. Particular attention is directed to those women with superfeminine builds who in reality are not overweight, but who would like to lose a few pounds. There are several simple methods for determining whether such patients are truly overweight when the tables which designate the desired weight, according to the subject's sex, height and body build, leave some doubt. The "pinch test" (performed over the lower lateral chest area) that produces a deep pinch of less than 1 inch is particularly helpful in avoiding this pitfall. Similarly, physicians should not subject slightly overweight subjects in their early teens to intensive weight reduction. Overweight in this age group is usually self-limiting, particularly if the person comes from an emotionally stable environment. (5) The patient should understand that close medical supervision is highly important when any weight-reducing diet which contains less than 1,000 calories per day is undertaken. (6) Added precautions should be taken when patients with peptic ulcer, gout, diabetes mellitus and cirrhosis of the liver are subjected to intensive weight reduction.

SUMMARY AND CONCLUSIONS

The effective management of obesity continues to pose one of the greatest and most frequent challenges to practicing physicians. There remains a large segment of the obese population for whom the usual program does not suffice. This segment includes the following types of patients: (1) those with serious and progressive medical disorders in whom prompt weight reduction is mandatory, including patients with heart disease, diabetes mellitus, arthritis, recurrent thrombophlebitis, peripheral vascular insufficiency of the lower limbs and sciatic neuritis; (2) those requiring elective surgery within the near future in whom the massive panniculus poses a decidedly

increased hazard; and (3) those who have become discouraged after a number of futile attempts at weight reduction, with or without medical supervision.

This report is concerned with a well conceived food preparation (Metrecal) which has been utilized in the comprehensive long term management of these three groups. Metrecal is not a panacea, but is of considerable value in both the initiation and maintenance of marked weight loss without insulting the body's metabolic economy. The technics of administration, the side effects encountered, an analysis of the results and a comparison with previous regimens of weight reduction are reported herein.

The fifty-seven patients studied were highly motivated persons, who presented themselves with one or several serious medical disorders which were clearly being aggravated by the excessive weight. These patients suffered from a wide variety of medical disorders, including advanced stages of coronary and hypertensive heart disease, peptic ulcer, cirrhosis of the liver, diabetes mellitus and patients with extensive rheumatoid arthritis on steroid therapy. No serious complications were observed.

In this group, 21 per cent lost 20 pounds or more, and 48 per cent lost from 10 to 19 pounds. These results compare favorably with those of studies employing other regimens.

Significant decreases in the serum cholesterol have been documented. Deviation of the levels of hypoprothrombinemia (anticoagulant escape) in nine patients receiving long term anticoagulant therapy was not encountered. Neither gout nor the elevation of the levels of ammonia in the blood (in a cirrhotic patient with a functioning portacaval shunt) was precipitated.

ADDENDUM

In a further experience with Metrecal, comprising more than fifty additional obese patients, these results continue to be supported. "Anticoagulant escape" was not encountered in eleven of these patients who were receiving anticoagulants.

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Studies of Vitamin A Deficiency in Children

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AMONG THE clinical manifestations of malnutrition in Indian children, those attributable to deficiencies of protein and vitamin A are the most widespread. While a great deal of attention has been devoted in recent years to the study of the problem of protein malnutrition, the problem of vitamin A deficiency which is no less important and more easily preventable has not attracted the same interest. It has been our experience (in Coonoor and Hyderabad, South India) that patients with vitamin A deficiency account for 25 to 30 per cent of all cases of clinical malnutrition in children.

Although the clinical manifestations of vitamin A deficiency have been recognized and described, there are still many aspects of the problem which would appear to require elucidation. For example, the frequent association of clinical signs of vitamin A deficiency with kwashiorkor suggests a possible interrelationship between protein malnutrition and vitamin A deficiency. The relationship between dietary intake of vitamin A and carotene, the levels of vitamin A in the serum, and the incidence and severity of clinical manifestations of vitamin A deficiency would seem to merit further investigation. In this paper the results of investigations of these and some other aspects of the problem of vitamin A deficiency in children are presented.

MATERIAL AND METHODS

The material for this investigation consisted of 319 cases of vitamin A deficiency, observed in Coonoor over a five year period (1952 to 1956) and forty-nine cases studied in Hyderabad over an eight month period in 1959. The investigation included the following:

clinical examination of cases, a survey of dietary intake with special reference to vitamin A and carotene, estimation of serum vitamin A and carotene levels before and after treatment and determination of the *in vitro* destruction of vitamin A by the patients' lysed red blood corpuscles. In addition, data on the incidence of vitamin A deficiency, obtained from the records of the Niloufer Hospital, Hyderabad for the five year period of 1954 to 1958, have also been considered. The assessment of dietary intake was carried out using the oral questionnaire method.

Serum vitamin A and carotene were estimated spectrophotometrically as follows. The absorption at 460 mμ and the difference in absorption at 328 mμ before and after irradiation were taken as measures of the carotene and vitamin A, respectively. Three ml. of serum or plasma was hydrolyzed with alcoholic KOH and extracted with petroleum ether. The layer of petroleum ether was washed and dried under vacuum and taken up in cyclohexane. After the initial readings were taken on the cyclohexane solution, it was irradiated for half an hour and a second measure of absorbance at 328 mμ was taken. The calculations were made as usual, taking the conversion factors into account.

The time required for the irradiation and the lower limit of the method were determined with solutions of pure vitamin A. All the solvents were specially purified and a reagent blank was carried out with every set of samples. It was found that a concentration below 10 I.U. per 100 ml. could not be estimated properly. The correction of Morton and Stubbs gave values in agreement with the aforementioned procedure only in samples of serum which had a high concentration of vitamin A.

The destruction of vitamin A by hemolyzed red blood cells was determined by the following

City	No. of Patients	Age (per cent)					Sex (per cent)	
		Less than 1 Year	1 to 3 Years	3 to 5 Years	6 to 10 Years	Over 10 Years	Males	Females
Coonoor	319	0.2	17.9	39.0	31.4	11.5	58	42
Hyderabad	551	3.8	26.8	48.7	18.2	2.5	60	40

procedure. The erythrocytes were separated from oxalated blood and washed with saline. Four volumes of water were added and the lysis was completed by freezing and thawing. Five ml. of the lysed solution equivalent to 1 ml. of red blood cells was used. Concentrated emulsion of vitamin A acetate was prepared, according to Pollard and Bieri.[1] It was suitably diluted on the day of estimation so that 0.5 ml. of emulsion would be equivalent to about 25 I.U. of vitamin A. The lysed cells and 0.5 ml. of the emulsion were incubated at 37° c. Alcoholic KOH was added at the end of fifteen minutes. Vitamin A was extracted and estimated with antimony trichloride solution. The vitamin A concentration in the emulsion was checked at the time of each estimation. The reduction in the vitamin A content of the incubated sample was taken as the amount of vitamin A destroyed and expressed as a per cent of the actual amount incubated.

The estimations of serum vitamin A and carotene and the determination of the *in vitro* destruction of vitamin A by the patient's lysed red blood cells were carried out in cases of vitamin A deficiency studied in Hyderabad. The estimations were repeated after treatment in a number of cases. For purposes of comparison, similar estimations were also carried out in apparently normal children of the same age group and socio-economic status in Hyderabad and in children with kwashiorkor who did not show clinical signs of vitamin A deficiency. Data concerning the different groups of children from whom these estimations were made, their clinical condition and the plan of treatment adopted are included in this report. (See Table III.)

The treatment in cases of kwashiorkor without vitamin A deficiency signs (Group 2) consisted of the administration of a high protein diet without a vitamin A supplement (the protein being almost solely derived from skim milk to which no vitamin A was added). Subjects with kwashiorkor and with signs of vitamin A deficiency (Group 4) received a high protein diet and, in addition, supplements of vitamin A daily. The vitamin A deficiency observed in nine of the seventeen children in this group was of the severe type involving the cornea, necessitating parenteral (in preference to oral) administration of vitamin A. These patients received 200,000 U. of vitamin A parenterally each day for eight to ten days; they subsequently were given 90,000 U. of vitamin A orally each day for approximately a fortnight. The remaining eight persons in this group, showing only conjunctival signs of vitamin A deficiency, were treated orally. Thirteen of the subjects with vitamin A deficiency and without kwashiorkor in Group 3a exhibited only conjunctival manifestations while four had keratomalacia. Patients with conjunctival manifestations received oral treatment, while those with keratomalacia received 200,000 U. parenterally. All children in Group 3b presented conjunctival manifestations, only.

All patients studied were afebrile at the time of the investigation.

Incidence and Clinical Features

The age and sex incidence of the children with vitamin A deficiency are indicated in Table I, and the clinical features of the subjects studied (319 in Coonoor and forty-nine in Hyderabad)

TABLE II
Main Clinical Features of Vitamin A Deficiency in Children

City	Total Number of Patients	Clinical Features			
		Conjunctival Lesion Only	Night Blindness Only	Conjunctival Lesion and Night Blindness	Keratomalacia
Coonoor	319	113	85	98	23
Hyderabad	49	14	0	2	33

are given in Table II. The maximal incidence was observed in the age period between three and five years. Nearly 60 per cent of the subjects were boys and 40 per cent were girls. However, this difference in sex incidence may reflect the general attendance of the two sexes in the clinic and may possibly be related to the tendency of poor women to seek medical advice more promptly and frequently for their boys than for their girls. It may thus have no etiologic significance.

Of the 319 children observed in Coonoor, only twenty-three showed corneal involvement (7.2 per cent). In the great majority of patients, the signs of hypovitaminosis A were of the milder type, involving only the conjunctiva. Bitot's spots were encountered in 102 of the 211 subjects showing conjunctival manifestations, and were invariably bilateral and situated on the lateral side of the cornea. The remaining children showed conjunctival xerosis characterized by dryness, discoloration and wrinkling of the bulbar conjunctiva without Bitot's spots.

An interesting feature was the lack of association between the presence of night blindness and the incidence of visible signs in the eye of vitamin A deficiency. Eighty-five children had night blindness without any other clinical manifestation of vitamin A deficiency, for the conjunctiva and cornea were clear and normal. In 113 subjects showing conjunctival lesions, no history of night blindness could be obtained, even after leading questions were posed. Keratomalacia observed in twenty-three patients was invariably bilateral with one eye being more affected than the other, and included lesions conforming to both the types described by Oomen[2] (viz., "multiple erosions of the conjunctiva leading to mummification of the cornea but without loss of general shape of the cornea" and "colliquative necrosis of the whole cornea leading to shrinkage of the eye ball"). From the etiologic viewpoint and from the responses obtained through treatment, there seemed to be no clear-cut distinction between these two types as they appeared to be merely clinical variations related to the speed and the severity of the same disease.

Of the forty-nine patients studied in Hyderabad, thirty-three had the severe type of vitamin A deficiency involving the cornea. In only sixteen cases were the lesions of the conjunctival type. The preponderance of corneal involvement in this particular investigation is in contrast to the low incidence (7.2 per cent) of keratomalacia found in Coonoor. It was quite clear that the vitamin A deficiencies in the children of Hyderabad were of a more severe type than those observed in the children of Coonoor. The age of the subject did not appear to be a factor in determining whether the site of the lesion occurred on the conjunctiva or the cornea. A possible objection to the assessment of the incidence of keratomalacia, in relation to the total incidence of vitamin A deficiency, purely on the basis of hospital admissions might be that only persons with severe vitamin A deficiency may be admitted, while those with the milder conjunctival type may have received treatment as outpatients. To obviate this possible objection, the incidence of manifestations of vitamin A deficiency in a series of patients admitted to the hospital in Hyderabad for kwashiorkor was compared with a series of patients with kwashiorkor

TABLE III

Serum Carotene, Vitamin A and the *In Vitro* Destruction of Vitamin A by the Red Blood Cells in Different Groups of Children.

Group No.	Clinical Condition	Treatment	Serum Carotene* (ratio/100 ml.)		Serum Vitamin A (I.U./100 ml.)*				*In vitro* Destruction of Vitamin A* (per cent)	
					Before Treatment†		After Treatment†			
			Before Treatment	After Treatment	No. Below 10 I.U.	Mean	No. Below 10 I.U.	Mean	Before Treatment	After Treatment
1	Apparently normal	...	50 (6)	...	0	80 (6)	...	...	28 (4)	...
2	Kwashiorkor only	High protein diet with no vitamin A	22 (13)	19 (7)	5	52 (9)	0	71 (7)	28 (5)	...
3a	Vitamin A deficiency only	Vitamin A	18 (13)	26 (11)	6	32 (7)	0	102 (11)	52 (8)	30 (3)
3b	Vitamin A deficiency only	High protein diet with no vitamin A	12 (4)	11 (3)	2	45 (2)	2	59 (1)‡	62 (4)	79 (3)
4	Kwashiorkor with vitamin A deficiency	High protein diet with vitamin A	13 (14)	19 (9)	12	17 (2)	0	74 (10)	45 (4)	34 (2)

* The values given are the mean values of the number of samples indicated in parentheses. The number of patients after treatment includes some patients for which initial values were not available.

† The number of samples having a concentration of less than 10 I.U. have been indicated in this column, and the mean given in the next column is the average of values above 10 I.U.

‡ This particular sample had a concentration of 65 I.U. of vitamin A/100 ml. serum initially.

observed in Coonoor. The over-all incidence of vitamin A deficiency in the patients with kwashiorkor in both Hyderabad and Coonoor was between 32 to 36 per cent. However, while keratomalacia accounted for only 10 per cent of the children with vitamin A deficiency complicated with kwashiorkor in Coonoor, it accounted for 62 per cent, in Hyderabad. This indicates that the observed differences between the incidence of children with keratomalacia in Hyderabad and in Coonoor were real.

An examination of the monthly incidence of cases for a five year period failed to reveal any definite seasonal trend in both places. There was no correlation between the incidence of cases of vitamin A deficiency and of respiratory or alimentary disorders.

The Dietary Situation

An assessment of the dietary intake of carotene and vitamin A was carried out in a group of children with vitamin A deficiency in Hyderabad. A similar survey was also carried out using a control group of children of the same age composition and socio-economic status who did not show any signs of vitamin A deficiency. It was found that the vitamin A and carotene intakes of both the groups were considerably lower (250 to 300 I.U. of precursor and 100 to 150 I.U. of preformed vitamin A) than the recommended quantities. There was no significant difference between the control and vitamin A deficiency groups regarding carotene and vitamin A intake. It had been reported earlier that the intakes of carotene and vitamin A of patients with kwashiorkor in Hyderabad were not lower than those in Coonoor in spite of the fact that the incidence of keratomalacia was much higher in the former.[3]

It was apparent from these findings that

while vitamin A deficiency signs were always associated with a low dietary intake of carotene and vitamin A, the reverse was not true.   In a number of patients subsisting on low dietary intakes of carotene and vitamin A, no clinical signs of vitamin A deficiency could be detected. A closer examination of the diets of children with kwashiorkor also failed to reveal any striking difference (with regard to dietary vitamin A and carotene intake) between patients with and those without complicating vitamin A deficiency signs.

Serum Vitamin A and Carotene Levels

The values for levels of vitamin A and carotene in the serum of children in the four groups investigated in Hyderabad are included in Table III.   The levels of vitamin A in the serum of the apparently normal children (Group 1) were lower than the average values reported for normal children in other parts of the world.[2,4]   This finding was not unexpected, for although the children investigated in this group were free from disease, they were drawn from the poor socio-economic group subsisting on unsatisfactory diets.   As expected, the subjects with vitamin A deficiencies (Group 3) exhibited considerably lower levels of serum vitamin A than did those of Group 1.   It was interesting that patients with kwashiorkor with no signs of vitamin A deficiency (Group 2) also exhibited levels of serum vitamin A which were significantly lower than those observed in the children in Group 1.   The lowest levels of serum vitamin A were observed in the subjects exhibiting signs of both kwashiorkor and vitamin A deficiency (Group 4).   The values for serum carotene were uniformly low in all the groups.

After treatment with vitamin A, a marked increase in the levels of vitamin A in the serum was observed in all subjects with vitamin A deficiencies.   A striking observation was made of the children with kwashiorkor who exhibited low levels of serum vitamin A.   Treatment of these children with a high protein diet containing *no* vitamin A supplement brought about a significant increase in the levels of vitamin A in the serum.   It was apparent from these observations that protein malnutri-

tion can bring about a significant lowering of serum vitamin A, and treatment with a high protein diet without vitamin A supplementation can correct this.

In Vitro Destruction of Vitamin A

In apparently normal children studied in this investigation (Group 1), the *in vitro* destruction of vitamin A by the lysed red cells was approximately 28 per cent.   Subjects with kwashiorkor (Group 2) did not significantly differ from the normal children (Group 1) in this regard.   However, in children with vitamin A deficiencies with or without kwashiorkor (Groups 3 and 4), the extent of *in vitro* destruction of vitamin A appeared to be definitely of a much higher order than that of the normal subjects (Group 1).   However, there was no difference between Groups 3 and 4 which again demonstrated that the presence of kwashiorkor did not influence this function.

After treatment with vitamin A, it was noticed that the abnormally high destruction, *in vitro*, of vitamin A was lowered in regard to patients in groups 3 and 4 with vitamin A deficiencies.

COMMENTS

Dietary Intake

An important observation made in this study was that while the dietary intakes of vitamin A and carotene of children with vitamin A deficiencies were low, they were not significantly lower than those of other children of the same socio-economic group who did not show signs of vitamin A deficiency. This would suggest that in the development of vitamin A deficiency, factors other than the actual diets of the children at the time of the investigation also have to be considered. Although it is true that in a number of patients the onset of clinical signs of vitamin A deficiency was preceded by episodes of fevers, infections and alimentary disorders, such episodes were apparently no more frequent in children showing signs of vitamin A deficiency than in those of the same socio-economic group not suffering from this deficiency.   The time at which supplementary feeding was started

may be an important factor in this connection. Mothers in Coonoor started the supplementary feeding of their infants by the sixth month, while in Hyderabad supplementary feeding was initiated only after the end of the first year.[3,5] This would indicate that, although at the time of investigation the intake of vitamin A and carotene by the children in both places was nearly similar, the children of Hyderabad had been on a vitamin A deficient diet for a relatively longer period of time. This might partly account for the higher incidence of severe types of vitamin A deficiency in Hyderabad. The vitamin A content of the breast milk of poor Indian women in Coonoor has been shown to be about 70 I.U./100 ml.[6] The vitamin A content of the breast milk of poor mothers in Hyderabad is being determined at present. The preliminary indications are that the content of vitamin A in the latter is considerably lower than that observed in the women in Coonoor. Even assuming that the vitamin A content of milk in Hyderabad mothers is the same as in the Coonoor mothers and that the output of breast milk in the former women is as high as 600 ml. throughout the first year of motherhood, breast milk alone would provide only 400 I.U. of vitamin A to the infants.

Another important factor which might explain the lack of a direct relationship between the dietary intake of vitamin A by the children and the incidence of clinical vitamin A deficiency among them is the possible variation in the hepatic storage of vitamin A during their fetal periods. In human adults with presumably adequate hepatic storage of vitamin A, dietary deprivation of vitamin A, extending over several months, was necessary to produce significant depression in the levels of vitamin A in the serum.[7] The livers of newborn infants, whose mothers' intake of vitamin A is presumably adequate, have been found to contain considerable amounts of vitamin A.[8] A survey* of the diets of pregnant mothers in Hyderabad revealed gross deficiencies of vitamin A and carotene. The estimation of vita-

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* This survey, taken by Dr. X. X. Kalpakum, has not yet been published.

min A in the livers of stillborn infants and of those dying during the neonatal period (which is at present being attempted) will provide further information on this subject.

It may, however, be appropriate to emphasize the fact that no case of vitamin A deficiency was encountered, in this study, in a child with a really satisfactory intake of vitamin A and carotene.

Night Blindness and Other Signs

A lack of association between the incidence of night blindness and other ocular signs of vitamin A deficiency was observed in our study. The incidence of night blindness may be modified by such factors as the degree of exposure to sunlight,[9,10] the degree of associated anemia[11] and possibly the nutritional status with regard to riboflavin[12] and vitamin C.[13,14]

Protein Malnutrition and Vitamin A Deficiency

The question of the relationship between protein malnutrition and vitamin A deficiency has attracted some attention in recent years.[2] In our investigation, it was observed that serum vitamin A levels were significantly lowered in patients with kwashiorkor who showed no clinical evidence of vitamin A deficiency; and furthermore, that treatment with a high protein diet without a vitamin A supplement brought about an increase in the levels of vitamin A in the serum in these patients. It was also found that subjects with a vitamin A deficiency complicated with kwashiorkor had significantly lower levels of serum vitamin A than did those uncomplicated without kwashiorkor, or vitamin A deficiency. These observations might suggest that protein malnutrition might aggravate vitamin A deficiency. Arroyave et al.[15] found that the absorption of vitamin A was impaired in patients with kwashiorkor. The increase in the levels of vitamin A in the serum, brought about in subjects with kwashiorkor by a high protein diet (in this study), indicate that the effect of protein malnutrition may be an impairment of the mobilization of vitamin A stored in the liver. It was demonstrated that when there is no manifest protein malnutrition in a child deficient in vitamin A,

the administration of a high protein diet does not result in an increase in the levels of vitamin A in the serum. The significance of lower levels of vitamin A in the serum of patients with kwashiorkor requires further study.

Contrary to the suggestion that protein depletion may aggravate vitamin A deficiency, Jagannathan[16] found that increasing the amount of protein in the diet beyond a certain point had a hastening effect on the depletion of the stores of vitamin A in experimental animals. He also observed an inverse relationship between growth in young rats and hepatic storage of vitamin A. The work of McClaren[17] indicated that protein depletion actually delayed the development of signs of vitamin A deficiency in rats fed diets deficient in vitamin A. During our study, it was observed that six children with kwashiorkor, who had no signs of vitamin A deficiency on admission, developed conjunctival signs of vitamin A deficiency after a few weeks of treatment with a high protein diet. We have frequently observed patients with severe keratomalacia without any evidence of kwashiorkor and patients with kwashiorkor with absolutely no clinical evidence of vitamin A deficiency.

The apparently contradictory observations of the relationship between protein depletion and vitamin A deficiency can be explained if it is recognized that protein depletion may, on the one hand, aggravate vitamin A deficiency by interfering with the absorption of vitamin A and possibly with the mobilization of vitamin A from the liver, and, on the other hand, mitigate vitamin A deficiency by sparing the tissue requirement of vitamin A by inducing growth retardation. The degree of protein depletion may well determine the direction of the net effect.

Serum Vitamin A and Carotene Levels

In the subjects with vitamin A deficiency, both the serum vitamin A levels as well as the serum carotene levels were low. It would thus appear that defective conversion of carotene into vitamin A was not a factor in the development of this disease. The lowest value for the levels of vitamin A in the serum observed in an apparently normal child (without any

evidence of vitamin A deficiency) was 45 I.U./100 ml. The highest level of vitamin A in the serum observed in the series of patients investigated with uncomplicated vitamin A deficiencies was 65 I.U./100 ml.

We have observed that the state of protein nutrition may also be an important factor in determining the levels of vitamin A in the serum in a malnourished population. The results of the present investigation do not permit any conclusions as to the critical level of serum vitamin A values below which it could be considered to be of definite pathologic significance. However, it was apparent that in children with vitamin A deficiency but without complicating kwashiorkor, values for serum vitamin A below 50 I.U. were the general rule.

In Vitro Destruction of Vitamin A

The work of Kon et al.[18] indicated the possible presence of factors which cause destruction of vitamin A in rabbits and rats. Pollard and Bieri[1] showed that the lysed red blood cells of rats, in vitro, were most active in destroying vitamin A. In our study, we observed that the lysed red cells from the blood of patients with vitamin A deficiencies were much more potent in bringing about in vitro destruction of vitamin A than were those of normal subjects. Furthermore, we were able to correct this abnormality by treating these patients with vitamin A. Additional investigation is necessary in order to decide to what extent this observation is applicable to the in vivo state. The significance of this observation from the point of view of the pathogenesis of manifestations of vitamin A deficiency remains to be elucidated. The results obtained through treatment indicate that the capacity for increased in vitro destruction of vitamin A by the lysed red blood cells of patients with vitamin A deficiency is the result rather than the cause of vitamin A deficiency.

SUMMARY

The clinical features of 319 children with vitamin A deficiency observed in Coonoor and forty-nine children studied in Hyderabad have been discussed in detail. Estimations of the contents of vitamin A and carotene in the serum

and the *in vitro* destruction of vitamin A by lysed red blood cells were carried out.

There was a lack of association between the incidence of night blindness and other ocular signs of vitamin A deficiency. Although signs of vitamin A deficiency were always associated with a low dietary intake of carotene and vitamin A, the reverse did not always occur. The proportion of patients with vitamin A deficiency who had keratomalacia was much greater in Hyderabad than in Coonoor.

Levels of vitamin A and carotene in the serum were low in all children with vitamin A deficiency. Levels of vitamin A in the serum of subjects with kwashiorkor but without clinical signs of vitamin A deficiency were also found to be significantly lower than the levels in apparently normal children. Treatment with a high protein diet without vitamin A supplementation brought about a significant increase in the levels of vitamin A in the serum in the latter.

The *in vitro* destruction of vitamin A by lysed red blood cells of children suffering from vitamin A deficiency appeared to be higher than that of the normal controls Treatment with vitamin A lowered this abnormal *in vitro* destruction.

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Riboflavin in Red Blood Cells in Relation to Dietary Intake of Children

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THE LEVEL of riboflavin in red blood cells has been suggested as a sensitive and practical index for evaluating the riboflavin nutrition of the individual[1] although there have been few reports in the literature on the use of this technic. There is evidence that in severe dietary deficiency the level of riboflavin in red blood cells is lowered.[1,2] However, there is a paucity of data on well nourished individuals.

MATERIAL AND METHODS

In order to increase our understanding of the growth and development of a group of healthy children enrolled in the Child Research Council, an investigation of dietary intake was started in 1946; determinations of riboflavin in plasma and red cells were added to the program of blood study in 1948. Determinations of plasma riboflavin were discontinued in 1954 when analysis of the data showed that under the conditions of this study these determinations added little to our understanding of the physiologic status of individual children. At that time there were indications that the riboflavin in red blood cells might be more meaningful, therefore this procedure was continued in order to provide more data for later re-evaluation.

The children enrolled in this study came from upper middle class families in the Denver area and were primarily of North European extrac-

tion. Examinations were conducted at stated intervals from the day of birth to evaluate the physical, physiologic and psychologic status. No therapy was given by the Council staff. Nutrition histories were taken at monthly intervals during the first year of life and at intervals of three months thereafter. Nutrient intake was calculated from tables on food values. Details of this technic have been published.[4]

The method for determination of riboflavin in red blood cells by Burch, Bessey and Lowry[5] was used during an eleven year period by five different workers. The principle of the method is the estimation of riboflavin by the measurement of its fluorescence before and after reduction. No important changes have been made in the method as originally described but, because of variations in power supply, it has been found advisable to prepare a new standard curve for each day's analyses. In most cases determinations were made in duplicate or triplicate. Duplicate analyses have agreed within 5 to 15 per cent. This degree of precision seems insufficient for longitudinal studies since apparent individual variations may be due to errors inherent in the technic rather than to true differences in the state of riboflavin nutrition. An important theoretic source of error is the possibility that red blood cells may contain substances other than riboflavin whose fluorescence depends on the state of oxidation.

Blood was drawn for study at monthly intervals from three days to three months, at three-monthly intervals from three months to three years and thereafter at intervals of six months; determinations of riboflavin in red blood cells were not always done at each age on each child. The 25, 50 and 75 percentiles,

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TABLE I

Red Cell Riboflavin (μg. %)

Age	Boys						Girls					
	No. of Cases	Minimum	25 Per Cent	50 Per Cent	75 Per Cent	Maximum	No. of Cases	Minimum	25 Per Cent	50 Per Cent	75 Per Cent	Maximum
3 days ±	12	21.4	25.4	29.8	32.4	37.0	15	23.0	27.4	29.1	33.1	46.0
1 mo.	17	14.9	27.8	31.2	34.5	46.0	16	22.0	27.6	29.8	34.2	37.0
2 mo.	11	21.1	30.0	33.4	37.0	41.0	14	20.0	27.7	30.4	34.8	35.3
3 mo.	14	25.4	32.4	34.8	39.0	26.0	12	26.0	27.8	30.6	35.2	40.3
6 mo.	15	27.7	29.4	32.3	36.6	47.6	14	23.7	27.8	31.2	35.7	38.4
9 mo.	17	21.0	28.0	30.9	35.2	42.8	16	21.9	27.6	31.3	35.7	46.8
1 yr.	16	25.1	27.1	30.0	34.2	46.0	12	26.1	27.1	30.7	35.1	46.2
1¼ yr.	13	19.3	26.4	29.3	33.3	51.0	13	23.5	26.4	29.6	33.8	33.8
1½ yr.	18	18.2	25.7	28.6	32.4	37.0	13	19.5	25.4	28.7	32.8	33.3
1¾ yr.	14	17.9	25.1	28.1	31.8	27.3	18	18.6	24.4	28.0	32.0	33.0
2 yr.	12	19.0	24.7	27.7	31.3	35.4	17	17.5	23.7	27.3	31.3	35.0
2¼ yr.	12	21.4	24.3	27.2	30.8	34.3	17	17.9	23.1	26.8	30.7	36.4
2½ yr.	13	21.0	24.0	26.9	30.3	34.4	16	17.3	22.6	26.4	30.2	33.4
2¾ yr.	10	21.7	23.6	26.6	29.9	36.4	18	15.1	22.0	26.0	30.1	29.7
3 yr.	12	21.8	23.3	26.2	29.6	32.2	15	18.7	21.9	25.6	29.9	30.5
3½ yr.	14	18.0	22.7	25.7	28.9	27.8	18	19.3	21.3	25.0	29.6	38.6
4 yr.	12	20.3	22.3	25.3	28.3	30.0	16	16.0	20.9	24.5	29.4	42.0
4½ yr.	7	22.9	22.0	24.9	27.9	29.9	18	16.9	20.6	24.1	29.3	34.8
5 yr.	9	19.8	21.8	24.8	27.6	28.0	14	18.6	20.4	23.8	29.2	34.4
5½ yr.	18	16.9	21.7	24.7	27.2	29.4	17	15.9	20.3	23.7	29.1	35.5
6 yr.	17	13.4	21.7	24.7	27.2	32.2	15	17.0	20.2	23.6	29.1	36.6
6½ yr.	14	19.0	21.6	24.5	27.2	31.9	16	14.5	20.2	23.6	29.0	34.2
7 yr.	13	18.2	21.5	24.4	27.0	31.9	14	13.8	20.2	23.6	29.0	29.3
7½ yr.	7	15.8	21.4	24.2	27.0	29.0	15	17.6	20.2	23.5	29.0	29.3
8 yr.	8	17.4	21.3	24.2	27.0	30.1	14	14.6	20.2	23.4	28.9	34.2
8½ yr.	8	14.7	21.3	24.0	27.0	24.2	16	14.6	20.2	23.4	28.8	32.7
9 yr.	11	18.8	21.2	24.0	27.0	38.8	14	15.0	20.2	23.3	28.5	32.0
9½ yr.	9	16.1	21.2	24.0	27.0	27.1	11	15.2	20.2	23.3	28.2	26.1
10 yr.	15	16.1	21.2	24.0	27.0	36.3	11	17.2	20.1	23.2	27.9	31.8
10½ yr.	7	21.0	21.1	23.7	27.0	29.0	12	17.9	20.1	23.2	27.8	34.0
11 yr.	7	20.4	21.0	23.6	26.9	42.3	23	12.2	20.1	23.1	27.7	35.7
11½ yr.	8	18.8	20.9	23.4	26.8	39.6	22	17.0	20.1	23.1	27.6	32.6
12 yr.	19	16.3	20.8	23.1	26.6	36.6	17	16.4	20.1	23.0	27.5	28.0
12½ yr.	14	13.1	20.6	22.9	26.3	34.0	15	17.1	20.1	23.0	27.4	31.9
13 yr.	9	16.3	20.4	22.7	26.0	29.8	15	17.9	20.0	22.9	27.3	26.8
13½ yr.	12	18.1	20.3	22.3	25.7	29.2	21	12.2	20.0	22.8	27.2	30.5
14 yr.	14	15.2	20.2	21.9	25.3	28.5	20	17.2	20.0	22.8	27.1	30.4
14½ yr.	17	16.0	20.0	21.7	25.0	27.2	18	13.9	20.0	22.7	27.0	32.4
15 yr.	17	16.9	19.9	21.5	24.6	32.4	11	14.5	..	22.7	..	28.0
15½ yr.	14	13.9	19.8	21.4	24.2	32.8	2	19.4	..	22.7	..	27.1
16 yr.	13	12.8	19.7	21.3	23.8	38.0	12	16.4	..	22.7	..	29.7
16½ yr.	7	19.9	19.5	21.2	23.4	27.4	3	15.4	..	22.7	..	22.9
17 yr.	14	16.6	19.2	21.1	23.0	27.7	7	19.4	..	22.7	..	27.3

which have been visually smoothed, and the observed minimum and maximum values from three days to seventeen years of age are presented in Table I. Only those children were excluded who were known to have a serious illness at the time of the examination. As indicated in Table I, the number of determinations at each age is relatively small; no attempt was made to establish quartile levels on the infrequent values after seventeen years of age. However, these data are more extensive than any previously reported in the literature.

Because the determinations of riboflavin in red blood cells were done on a larger number of children but with less regularity than nutri-

tional histories, the data used for correlations are those on children for whom there were concomitant determinations in both areas of study. The dietary figures represent the average daily intake during the preceding month or three months or six months, the time interval increasing as the child becomes older; the blood level was determined at the end of that time interval. There have been 282 pairs of determinations on thirty-two boys and 334 pairs on thirty-six girls, ranging from one month to eighteen years of age.

RESULTS AND COMMENTS

Total dietary intake increases with age while the riboflavin in red blood cells, after a rise

TABLE II
Intake of Riboflavin (µg./kg. Body Weight)

Age (Year and Month)	Boys						Girls					
	No. of Cases	Mini-mum	25 Per Cent	50 Per Cent	75 Per Cent	Maxi-mum	No. of Cases	Mini-mum	25 Per Cent	50 Per Cent	75 Per Cent	Maxi-mum
0-0 to 0-1	20	91	197	237	277	334	19	152	207	242	276	305
0-1 to 0-2	24	136	240	281	327	338	24	109	224	278	307	376
0-2 to 0-3	26	125	200	248	278	340	24	50	217	250	278	336
0-3 to 0-4	27	160	192	222	243	306	27	51	211	238	267	307
0-4 to 0-5	28	141	185	212	235	284	29	67	202	228	253	372
0-5 to 0-6	28	124	178	208	229	279	31	58	192	218	236	348
0-6 to 0-9	28	145	170	198	221	265	34	142	176	200	216	332
0-9 to 1-0	32	122	156	184	208	236	34	131	155	176	198	270
1-0 to 1-3	31	86	137	165	185	256	34	87	140	160	186	247
1-3 to 1-6	30	82	118	141	165	248	34	88	128	147	175	211
1-6 to 1-9	28	55	98	128	148	248	34	83	118	134	163	207
1-9 to 2-0	28	46	91	116	140	213	36	59	108	123	150	198
2-0 to 2-3	27	46	87	110	135	207	35	45	102	116	135	197
2-3 to 2-6	28	64	86	107	132	177	34	67	95	109	125	170
2-6 to 2-9	28	40	85	104	130	184	31	74	89	103	118	144
2-9 to 3-0	27	64	84	103	129	181	31	56	85	99	114	167
3-0 to 3-3	27	58	83	101	128	204	31	56	81	96	110	148
3-3 to 3-6	28	68	82	100	126	178	31	53	79	94	108	138
3-6 to 3-9	26	39	81	99	124	163	32	43	77	93	107	141
3-9 to 4-0	25	61	80	98	121	162	29	43	76	92	106	135
4-0 to 4-3	26	59	80	97	118	170	29	53	75	91	106	134
4-3 to 4-6	26	59	80	96	115	172	27	42	75	90	106	134
4-6 to 4-9	27	54	79	95	112	153	27	57	74	89	105	123
4-9 to 5-0	26	53	79	94	110	149	27	48	73	88	105	120
5-0 to 5-3	27	52	79	93	108	129	27	44	72	88	105	181
5-3 to 5-6	26	58	78	92	107	151	27	37	71	87	104	181
5-6 to 5-9	26	56	78	91	106	136	26	54	70	87	103	151
5-9 to 6-0	26	65	77	90	106	140	24	50	70	86	102	151
6-0 to 6-3	26	58	77	89	105	127	24	56	70	85	101	148
6-3 to 6-6	24	63	76	88	104	127	24	54	70	84	99	127
6-6 to 6-9	23	62	75	87	103	130	25	56	70	83	97	128
6-9 to 7-0	23	59	74	86	102	113	26	43	68	81	96	122
7-0 to 7-3	22	54	73	85	100	110	27	53	67	80	94	117
7-3 to 7-6	20	54	72	84	99	107	27	51	65	79	92	117
7-6 to 7-9	18	44	70	83	98	115	28	45	64	78	90	128
7-9 to 8-0	17	52	69	82	97	128	28	42	62	76	88	115
8-0 to 8-3	17	53	..	81	..	109	26	49	61	74	85	111
8-3 to 8-6	16	57	..	80	..	112	25	44	59	73	83	108
8-6 to 8-9	17	48	..	79	..	112	24	47	57	71	81	96
8-9 to 9-0	17	53	..	78	..	113	23	45	56	69	79	107
9-0 to 9-3	14	55	..	77	..	102	23	45	54	67	77	100
9-3 to 9-6	13	41	..	76	..	116	22	43	53	65	75	98
9-6 to 9-9	12	45	..	75	..	107	23	38	51	63	74	91
9-9 to 10-0	12	52	..	74	..	108	22	38	49	62	73	91

in the first three months of life, decreases rapidly to six years and then more slowly throughout this age span. As a result, a negative correlation was found between the red cell level and total dietary intake of riboflavin. Therefore, dietary intake per kilogram of body weight was used for all further correlations. The smoothed percentiles and observed extremes of dietary intake of riboflavin per kilogram of body weight from birth to ten years of age are presented in Table II. Data over ten years are still inadequate in number and will not be presented here.

Since vitamin supplements were taken by some children in varying amounts, these were added to dietary intake for the initial correlations. The correlation coefficients for riboflavin in red blood cells and intake per kilogram from dietary sources plus vitamin supplements ($+0.44 \pm 0.06$ for boys and $+0.42 \pm 0.05$ for girls) were slightly lower than those for red cell level and intake per kilogram from dietary sources only. Since this is a group of healthy children whose intakes of total riboflavin[6] tend to be higher than the Recommended Allowance of the National Research Council,[7] it may be assumed that intake of riboflavin from diet alone is adequate for physiologic needs and that supplementation by vitamin preparation is superfluous. Further analysis
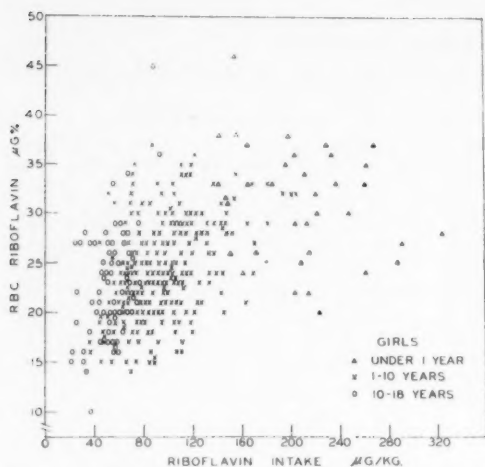
FIG. 1. Values for riboflavin in the red blood cells of girls of various ages (see text).

FIG. 2. Graphs for three children represent values obtained for both red cell riboflavin and dietary intake per kg. of weight, plotted on the rectilinear percentiles for age.

of the data was done with the exclusion of the supplements.

When red cell level was compared to dietary intake of riboflavin per kilogram of body weight, the correlation coefficients were $+0.47$ for boys and $+0.46$ for girls if all ages from one month to eighteen years were included. Inspection of the data showed that infants tend to have higher levels of riboflavin in both dietary intake and red blood cells while children over ten years of age tend to have lower levels. This may be seen in the graph of values for girls (Fig. 1) and suggests an artifact due to the similarity of decrease with age of both of these factors rather than a real effect of dietary intake upon the blood level. When smaller age groups were analyzed, the correlations between dietary intake and blood

level ranged from zero to $+0.39$ (Table III), indicating relatively little relationship. A wide range of red cell values was observed at each dietary intake level while a wide range of dietary intakes was found in children whose blood values were similar.

Analysis of the records of individual children showed no consistent relationship between dietary intake and the level of riboflavin in red blood cells. As with the group data, there was greater disparity in blood level of the individual when vitamin supplements were added to diet than when dietary intake alone

TABLE III

Correlation Coefficients of Dietary Intake of Riboflavin per Kilogram of Body Weight with Red Blood Cell Riboflavin

Age	Boys		Girls	
	Correlation Coefficient	No. of Cases	Correlation Coefficient	No. of Cases
Under 1 yr.	$+0.14 \pm 0.12$	65	0 ± 0.15	41
1–6 yr.	$+0.18 \pm 0.08$	140	$+0.26 \pm 0.08$	154
6–10 yr.	$+0.07 \pm 0.14$	53	$+0.30 \pm 0.12$	71
10–18 yr.	$+0.05 \pm 0.20$	24	$+0.39 \pm 0.12$	68
Total Group	$+0.47 \pm 0.06$	282	$+0.46 \pm 0.05$	334

was considered. An attempt to determine whether there might be a level of intake which could be interpreted as a saturation level in relation to the blood values was fruitless. The inconsistencies between intake and blood levels were so great that one child with a fairly constant intake level might show wide fluctuations in the level of riboflavin in red blood cells or another child with marked changes in intake might have fairly constant red cell values. Examples of the findings in three children are shown in Figure 2, in which values for both red cell riboflavin and dietary intake per kilogram of weight are plotted on the rectilinear percentiles for age.

Basal metabolic rates and patterns of growth in height and weight seem to bear little relationship to the levels of riboflavin in red cells and were not helpful in explaining the discrepancies between intake and blood levels. No significant relationship was found between riboflavin levels in red blood cells and reticulocyte counts of fifty-eight children under ten years of age.

CONCLUSION

It must be concluded that in this group of healthy children, although both dietary intake of riboflavin per kilogram of body weight and the riboflavin level in red blood cells decrease with age, the level of dietary intake seems to bear no significant relationship to the level of riboflavin in red blood cells within the intake range observed.

SUMMARY

As part of a study of the growth and development of children, determinations of riboflavin in red blood cells and in the diet were done at stated intervals for several years on the same children. This paper presents the quartile values and the minimum and maximum levels of riboflavin in red blood cells and of the dietary intake of riboflavin per kg. of body weight. A correlation coefficient of $+0.47$ was found in 282 paired determinations on thirty-two boys and a coefficient of $+0.46$ in 334 paired determinations on thirty-six girls, ranging from one month to eighteen years of age. Infants tend to have higher levels of riboflavin in both dietary intake and red blood cells while children over ten years of age tend to have lower levels. Within each age group correlations indicate relatively little relationship between the dietary intake of riboflavin and the amount of that substance found in the red blood cells.

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Trace Metals in Human Plasma and Red Blood Cells

A Study of Magnesium, Chromium, Nickel, Copper and Zinc
I. Observations of Normal Subjects

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A T LEAST thirty-seven of the 102 chemical elements have been found in the human body. Many of the thirty-seven elements, of which twenty-six are metals, are present in only trace amounts, and several of them are sometimes either absent or present in amounts too small to detect by the methods of analysis used. It is probable that traces of other elements will be found in the human body when more sensitive methods have been developed.‖

Most of the elements found in the tissues and body fluids are also present in the blood. As our knowledge increases it is becoming apparent that many of the metallic elements have a

specific metabolic function and that their presence is not merely the result of contamination from our environment. In most cases, proof that their presence is essential is lacking in spite of cogent evidence for a specific role. This is partly due to our failure to observe clear-cut states of deficiency in man and partly due to the difficulties in conducting appropriate experiments *in homo vivo*. Experiments with animals suggest the probable mechanisms of the metallic elements but how well these data may be applied to man is problematical, since there may be differences in the components involved in corresponding reactions. The non-specificity of some metals for certain reactions is another complicating factor.

Biologically active metals usually exert their effects through enzyme systems. There are two classes of such enzymes: (1) metalloenzymes, which have a fixed amount of specific metallic ion per molecule of protein—addition of an agent which binds the metal inactivates the enzyme, often irreversibly; (2) metal-protein complexes, which are a larger group of proteins loosely bound to metal. Metals may substitute for each other with varying degrees of affinity and they may be removed by dialysis. Some of these complexes have enzymatic activity while others are probably concerned with transport only.[1] Combination in both types of enzymes is probably effected through chelation.[2,3]

After the development in the Pratt Trace Analysis Laboratory of an accurate procedure

TABLE I
Levels of Magnesium, Chromium, Nickel, Copper and Zinc in Normal Plasma
(Mean and ranges in p.p.m.[*])

Source	Method	Metal	Range	Mean	No. of Samples[†][*]
Monacelli et al.[4]	Spectrochemical	Magnesium[‡]	10.0–42.0	24.0	25
Albritton[20]	Colorimetric	Magnesium	17.0–28.8	20.4	. . .
Paixao and Yoe[5]	Spectrochemical	Magnesium	13.1–37.2	19.4	39
Present study	Spectrochemical	Magnesium	12.5–36.0[§]	20.4	109
Monacelli et al.[4]	Spectrochemical	Chromium	0.08–0.30	0.180	25
Koch et al.[23]	Spectrochemical	Chromium	0.007–0.052	0.022	17
Paixao and Yoe[5]	Spectrochemical	Chromium	0.016–0.038	0.024	39
Present study	Spectrochemical	Chromium	0.009–0.055	0.027	109
Monacelli et al.[4]	Spectrochemical	Nickel	0.01–0.06	0.040	12
Koch et al.[23]	Spectrochemical	Nickel	0.01–0.09	0.030	. . .
Paixao and Yoe[5]	Spectrochemical	Nickel	0.00–0.18	0.023	39
Present study	Spectrochemical	Nickel	0.00–0.27	0.060	109
Monacelli et al.[4]	Spectrochemical	Copper	1.00–2.10	1.20	22
Koch et al.[23]	Spectrochemical	Copper	0.67–1.30	0.92	12
	Colorimetric	Copper	0.65–1.35	0.98	58
Cartwright[24]	Colorimetric	Copper	. . .	1.05	12 M
	Colorimetric	Copper	. . .	1.14	11 F
Paixao and Yoe[5]	Spectrochemical	Copper	0.50–2.47	0.99	39
Present study	Spectrochemical	Copper	0.48–1.93[§]	1.03	109
Monacelli et al.[4]	Spectrochemical	Zinc	0.60–2.30	1.30	14
Albritton[20]	Colorimetric	Zinc	0.00–6.30	3.00	. . .
Koch et al.[23]	Colorimetric	Zinc	0.32–1.70	1.21	26 M
	Colorimetric	Zinc	0.79–1.50	1.19	32 F
Wolff (by Koch)[23]	Colorimetric	Zinc	1.37–2.84	1.97	50 M
	Colorimetric	Zinc	1.41–2.72	1.94	50 F
Paixao and Yoe[5]	Spectrochemical	Zinc	0.48–4.80	2.70	39
Present study	Spectrochemical	Zinc	0.49–7.70[§]	3.01	109

* Parts per million (μg. per gm.) is used throughout this report.

† Where indicated, M = male subjects and F = female subjects.

‡ There are many reports in the literature on colorimetric methods which have yielded values indicating a serum range of 17 to 30 p.p.m.,[21] but data on erythrocytic levels are few.

§ Since Paixao and Yoe's paper was published, four of their control subjects have been found to have evidence of disease. These four are not included in this series, which explains apparent discrepancies between their ranges and those of the present study.

for determining trace metals we began this study in an effort to define any correlations which may exist between states of disease and alterations the levels of certain metallic microelements in the blood. This study has been a cooperative effort between the Department of Internal Medicine of the University of Virginia School of Medicine and the Pratt Trace Analysis Laboratory. The first part of our study, reported herein, is concerned only with observations made on normal blood; observations of the blood of patients with various hematologic diseases are reported in the article which follows.

The metals studied are magnesium, chromium, nickel, copper and zinc. The method employed is spectrochemical and is described by Monacelli, Tanaka and Yoe[4] and Paixao and Yoe.[5] The equipment and the procedures of collection which were employed are also described in these publications. The normal subjects were medical students, physicians and employees of the University of Virginia.

In Tables I and II, plasma and red cell values are listed for the respective metals as published by other investigators, for comparison with our own data. The data recently reported by Paixao and Yoe[5] have been incorporated into the present series. Unlike other results reported ours were analyzed for the possible influence of age, sex and race. Since no important trends were observed,

TABLE II

Levels of Magnesium, Chromium, Nickel, Copper and Zinc in Normal Red Blood Cells

(Mean and ranges in p.p.m.)

Source	Method	Metal	Range	Mean	No. of Samples*
Albritton[20]	Colorimetric	Magnesium	. . .	61.2	. . .
Paixao and Yoe[5]	Spectrochemical	Magnesium	26.6–112.0	64.8	40
Present study	Spectrochemical	Magnesium	26.0–131.0	74.3	106
Paixao and Yoe[5]	Spectrochemical	Chromium	0.011–0.041	0.022	40
Present study	Spectrochemical	Chromium	0.005–0.054	0.021	106
Paixao and Yoe[5]	Spectrochemical	Nickel	0.00–0.16	0.051	40
Present study	Spectrochemical	Nickel	0.00–0.31	0.053	106
Koch et al[23]	Colorimetric	Copper	0.21–1.97	0.85	60
Cartwright[34]	Colorimetric	Copper	. . .	0.76	12 M
	Colorimetric	Copper	. . .	0.74	11 F
Paixao and Yoe[5]	Spectrochemical	Copper	0.28–3.50	0.94	40
Present study	Spectrochemical	Copper	0.28–2.80†	0.82	106
Albritton[20]	Colorimetric	Zinc	9.10–19.70	14.40	. . .
Koch et al.[23]	Colorimetric	Zinc	8.26–14.85	12.06	25 M
	Colorimetric	Zinc	7.65–15.0	11.57	32 F
Wolff (by Koch)[23]	Colorimetric	Zinc	. . .	12.95	10 M
	Colorimetric	Zinc	. . .	13.00	10 F
Paixao and Yoe[5]	Spectrochemical	Zinc	3.80–16.60	11.40	40
Present study	Spectrochemical	Zinc	3.80–25.40	10.00	106

* See footnote † to Table I.
† See footnote § to Table I.

TABLE III

Analysis of Data According to Sex

(Mean Values in p.p.m.)

Sex	No. of Samples	Mg	Cr	Ni	Cu	Zn
Plasma						
Male	92	20.6	0.027	0.063	1.00	3.14
Female	17	20.1	0.025	0.046	1.17	2.3
Average	109	20.4	0.027	0.060	1.03	3.01
Red Blood Cells						
Male	89	75.9	0.022	0.057	0.83	10.5
Female	17	66.9	0.016	0.036	0.81	7.0
Average	106	74.3	0.021	0.053	0.82	10.0

graphs of these analyses were not included. In some age groups the number of samples analyzed is small, and for this reason an effect from aging cannot be excluded. However, it is apparent from our own results that there are no significant differences between male and female subjects and between Caucasians and Negroes (Tables III and IV). Red cell counts, hematocrit results, hemoglobin measurements and white cell counts of the blood of all subjects were found to be within normal range.

TABLE IV
Analysis of Data According to Race
(Mean Values in p.p.m.)

Race	No. of Samples	Mg	Cr	Ni	Cu	Zn
Plasma						
Caucasian	96	20.5	0.027	0.061	1.01	3.04
Negro	13	21.6	0.030	0.056	1.19	2.80
Average	109	20.4	0.027	0.060	1.03	3.01
Red Blood Cells						
Caucasian	93	74.7	0.022	0.054	0.83	10.3
Negro	13	71.4	0.017	0.038	0.87	7.0
Average	106	74.3	0.021	0.053	0.82	10.0

MAGNESIUM

The content of magnesium in the entire body of the average sized adult is about 25 gm. It is the fourth most abundant cation, being exceeded only by calcium, sodium and potassium.[6] Thus it is hardly correct to regard magnesium as a "trace element." In the intracellular fluid it is the third most common cation, being exceeded only by potassium (151 mEq. per L.) and sodium (15 mEq. per L.). It is estimated that there are 26 mEq. per L. of magnesium in the intracellular fluid, but numerically sodium ions are in greater abundance because of their univalence. In extracellular water its concentration is about one tenth of that in intracellular water, ranking it immediately behind potassium in terms of abundance.[7]

About half of the total magnesium in the body is in the skeleton and slowly exchanges with the extracellular ion. Liver and muscle contain the highest concentrations. The brain, kidneys and red cells also contain relatively large amounts.[8]

About 35 per cent of serum magnesium is bound to albumin and globulin while the remainder is ionized and accounts for most of its biologic activity.[7-9]

Magnesium performs a large number of functions. It activates many different enzymatic systems and in this way is probably involved in many major metabolic processes. Although this activation has been shown to have an *in vivo* counterpart in only a few instances, there is convincing evidence that magnesium is essential in human metabolism.[6] It is a depressant to both the central and peripheral nervous systems and plays a major role in the maintenance of normal neuromuscular excitability. Magnesium depresses cardiac conduction and peripheral vascular tone.[7] It is also required in the properdin system.[10]

The average daily intake of magnesium is about 300 mg. Estimates indicate that the requirement is not more than 250 mg. in the normal adult, 150 mg. in the infant, and 400 mg. in pregnancy. Two thirds of the normal intake of magnesium is not absorbed and appears in the stool. Concentrated solutions of magnesium induce catharsis through osmotic action and are less well absorbed than dilute solutions.[8,11] That which enters the circulation is excreted by the kidneys and may cause diuresis. Practically none is excreted by the liver. Magnesium is filtered at the glomerulus and normally 95 per cent is reabsorbed by the tubule.[12] The interaction of magnesium and potassium in the kidney is of interest. Administration of magnesium (and the urinary excretion thereof) may cause a decrease in the excretion of potassium,[12,13]

but the reverse does not occur. Diuretics
which decrease the excretion of potassium
increase the excretion of magnesium (e.g.,
ammonium chloride) and vice versa (e.g.,
acetozolamide). Aldosterone increases the
excretion of both potassium and magnesium,
and both are retained in renal failure.[7,8,14,15]
Administration of magnesium is also followed
by urinary excretion of calcium in proportion
to the level of magnesium in the serum,[13]
whereas intravenous calcium loading causes a
decrease in the content of magnesium in the
serum and the urine (probably resulting from
ingress into the cells).[16]

Magnesium and potassium are similar in
distribution. Like potassium, magnesium
tends to prolong the P R and Q T intervals of
the electrocardiogram.[17-19]

Plasma values for magnesium fall con-
sistently within a rather narrow range (Table
I). Our red cell values are uniformly dis-
tributed over a much wider range, with an
average amount three and one half times as
great as that of the plasma.

CHROMIUM

Little is known about the occurrence of
chromium in human tissues and as yet no
physiologic role has been attributed to this
element. Urone et al., using a colorimetric
method, made more than 1,000 determinations
on various body tissues, blood and urine.
They found the amount of chromium to vary
from undetectable amounts to 0.3 p.p.m.
in whole blood, 0.35 p.p.m. in urine, and 45
p.p.m. in tissues with the largest amounts
being in the lungs and kidneys.[22] Koch et al.
found chromium in all of the 17 samples of
normal plasma and in all the tissues examined
with the highest concentrations being in the
small bowel and thyroid.[23] In the Pratt Trace
Analysis Laboratory, chromium has been
consistently found in normal human plasma
and red blood cells.[4,5] Chromium has been
implicated in carcinogenesis.[24]

From inspection of Table I, it is evident
that our values for chromium are in close
agreement with those of Koch and his co-
workers.[23] The plasma levels found by
Monacelli et al. are much higher.[4]

NICKEL

So few data are available on the concen-
tration of nickel in body tissues and fluids
that normal values cannot be stated with
certainty. Koch et al. studied normal tissues
by spectrochemical analysis and found nickel
in most of the organs studied with the highest
concentrations being in the small bowel, bladder,
lung and cardiac muscle.[23] Tietz et al.
studied apparently unaffected tissues from the
lungs, liver and kidneys of patients with primary
and metastatic carcinomata, inflammations
and other conditions, and concluded that
normal ranges in these three tissues were 0.0
to 0.30, 0.0 to 0.20, and 0.0 to 0.15 mg. per
100 gm. of dry tissue, respectively.[24] The
validity of these results is debatable since
disease may have unrecognized effects on
apparently uninvolved tissues. Tietz also
stated that Araki and Mure reported a gradual
increase with age in the concentration of
nickel in tissue, giving an average tissue
value of 0.28 p.p.m. in persons up to thirty-
one years of age and 1.50 p.p.m. in persons over
fifty-one years (the age group with an ex-
pected higher incidence of carcinoma). Tietz's
results were not correlated with age.

Nickel may be found in the plasma. To the
best of our knowledge its presence in the red
blood cells was first demonstrated by Paixao
and Yoe.[5]

An essential function for nickel has not yet
been demonstrated, but neither has a serious
effort been made to find one. Such a discovery
would not be surprising since nickel bears a
close physical and chemical relationship to
cobalt, the essential metallic constituent of
vitamin B_{12}. *In vitro* nickel activates arginase,
carboxylase and trypsin, and it may inhibit
acid phosphatase.[25,26a] There is evidence that it
affects the clotting mechanism by stabilizing
the labile factor.[3]

On the basis of a limited study of two normal
men, Kent and McCance[26b] suggest that an
ordinary diet may supply 0.3 to 0.5 mg. of
nickel per day. This is probably highly
variable in view of the large amounts con-
tributed by metal containers and utensils.
More nickel was found in the urine than the
stool. Intravenously injected nickel chloride

was found to be slowly excreted, chiefly by the kidneys. The part recovered in the stool was presumed to be excreted by the liver.[25]

Table III shows our results to be higher than those of Monacelli et al.[4] and Koch et al.[23] In this range of extremely small quantities, slight absolute changes produce large variations in percentages. Another factor, reflected in the mean value of Paixao and Yoe,[5] is the large number of negative samples in their series. In our study, nickel was undetected in only eleven of 109 plasma samples. In only four of 106 red cell samples was there no nickel found. In only one person was nickel absent in both the cells and plasma examined. In Paixao and Yoe's study nickel was not detectable below 0.02 p.p.m. for red cells and 0.04 p.p.m. for plasma. The sensitivity of this method which was used in our study was improved (the new sensitivity being of the order of 0.007 p.p.m.) and the samples of Paixao and Yoe were reanalyzed by the new procedure before being included in our series.

The absence of nickel from the plasma or red cells of a few subjects may be regarded as evidence against the existence of a physiologic function for this element.

COPPER

It is estimated that the body contains 100 to 150 mg. of copper. Most of this is present in the liver (the chief organ of storage and excretion) and the central nervous system. The spleen and bones have the lowest concentrations of copper. Ninety-five per cent of that present in the plasma is firmly bound to an alpha-2 globulin.[27] Because of its blue color this complex was called "ceruloplasmin" by Holmberg and Laurell, its discoverers.[28] The remainder is loosely bound to other proteins, chiefly albumin. Copper in the red blood cells also occurs in two fractions, the loosely bound portion being in equilibrium with the corresponding plasma fraction. Transport into the cell apparently occurs in this form, and after a delay of eighteen to twenty-four hours the copper becomes firmly bound.[27] At least 80 per cent of erythrocytic copper occurs in the form of a colorless protein similar but not

identical to the hemocuprein of Mann and Keilin.[29] This fraction was isolated by Markowitz, who named it "erythrocuprein."[30] Copper-containing proteins react with diethyldithiocarbamate in "direct" and "indirect" manners, the firmly bound portion requiring intermediate cuprolysis with acid.[27,31]

Ceruloplasmin may act as an oxidase upon many substrates, including serotonin,[32] ascorbic acid and several polyphenols.[33] Other copper-containing enzymes are uricase and tyrosinase.[27] It has been shown that copper is necessary for optimal nutrition in animals, but the essential nature of this element in man has not been proved. It is probably involved in myelination of nervous tissue and in maintenance of normal skin pigmentation.[34] Its probable action in erythropoiesis is to assist in the absorption of iron and its incorporation into protoporphyrin in the formation of heme.[25,27]

The average adult ingests 2.5 to 5.0 mg. of copper daily. It is estimated that positive balance can be maintained on 2 mg. per day. Studies of Cu^{64} have shown 30 to 40 per cent absorption of orally administered 1 mg. doses. Ninety-nine per cent of orally or parenterally administered copper appears in the stool; thus the major portion of that absorbed is excreted via the bile. Zero to 70 μg. appear in the urine per day, with slight variations according to intake.[27,34]

Our data, concerning values for copper, closely agree with the reported values in Tables I and II. Our results agree with Cartwright's finding of no significant difference between the sexes. One red cell value (2.8 p.p.m.) is conspicuously high (next highest value is 1.6 p.p.m.) and accounts for the wide range. Average values are somewhat higher in the plasma. (Our average for the levels of copper in the red cells shows a decrease in the fifth decade and there is a small rise in the plasma value (Table v).

ZINC

The body of the average adult contains about 2.2 gm. of zinc.[1] Large amounts are present in the prostate (859 $\pm$ 96 p.p.m.)[35] and retina (500 to 1,000 p.p.m.) but most other

TABLE V
Averages for the Respective Age Groups*
(p.p.m.)

Age Group	10-20		20-30		30-40		40-50		10-60	
					Plasma					
No. of Samples:	12		56		26		13		109	
Metal	Mean	Spread	Mean	Spread	Mean	Spread	Mean	Spread	Mean	Spread
Mg	18.1	12.7–25.7	20.0	13.1–36.0	22.3	13.7–32.4	21.0	13.0–26.6	20.4	12.5–36.0
Cr	0.028	0.013–0.041	0.026	0.009–0.055	0.028	0.015–0.038	0.026	0.016–0.040	0.027	0.009–0.055
Ni	0.060	0–0.17	0.063	0–0.27	0.051	0–0.17	0.067	0–0.15	0.060	0–0.27
Cu	0.94	0.48–1.50	1.02	0.52–1.79	0.99	0.75–1.48	1.16	0.66–1.93	1.03	0.48–1.93
Zn	2.86	1.00–7.70	2.82	0.50–6.10	3.50	1.1–6.5	3.14	0.90–5.6	3.01	0.49–7.70
					Red Blood Cells					
No. of Samples:	11		56		25		13		106	
Metal	Mean	Spread	Mean	Spread	Mean	Spread	Mean	Spread	Mean	Spread
Mg	78.9	41.8–116.0	70.5	29.9–112.0	82.4	36.1–120'	70.4	26.0–131.0	74.3	26.0–131.0
Cr	0.022	0.011–0.054	0.021	0.006–0.041	0.023	0.010–0.039	0.019	0.007–0.030	0.021	0.005–0.054
Ni	0.034	0–0.10	0.054	0–0.17	0.062	0–0.18	0.053	0–0.16	0.053	0–0.31
Cu	0.88	0.48–1.31	0.86	0.33–2.8	0.84	0.46–1.56	0.63	0.28–0.94	0.82	0.28–2.8
Zn	9.1	3.8–25.4	9.9	3.8–20.9	11.0	4.2–20.6	9.3	3.8–17.0	10.0	3.8–25.4

* Two subjects were more than fifty years old. The two plasma values and one cell value obtained do not show as a separate age group but are included in the last column.

organs contain 20 to 30 p.p.m.[1] Using a colorimetric method Vallee found 8.8 ± 0.2 p.p.m. in whole blood.[36]

There are no seasonal or diurnal variations nor are there differences between the sexes in levels of zinc in the blood. The red cells contain by far the greatest portion of zinc in the blood but individual leukocytes contain about twenty-five times as much as the individual red cells. The content of erythrocytic zinc in the infant is one fourth that of the adult and reaches adult levels at ten to twelve years of age.[1]

About one third of the zinc in the serum is firmly bound to globulin. The remainder is loosely bound to albumin and is probably concerned with transport.[1] As the relationship of zinc to the human serum dehydrogenases is clarified, it may develop that a portion of the zinc in the serum is bound to these substances also.

Zinc appears to be an integral part of carbonic anhydrase since this enzyme is irreversibly inactivated when zinc is removed by binding agents.[1,37] Beef pancreas carboxypeptidase and several diphosphopyridine nucleotide (coenzyme I)-dependent dehydrogenases of yeast and animal origin have been shown to be metalloenzymes, requiring zinc for their activity.[38-40] It is likely that such enzymes account for much of the zinc in human blood, but to the best of our knowledge this has not been proved.

Eighty per cent of human leukocytic zinc is bound to a protein, the function of which is not known. Possibly lactic dehydrogenase accounts for some of the remainder.[41,42] Carbonic anhydrase is not found in these cells.[1]

The usual daily intake of zinc is 10 to 15 mg. About 4 per cent is excreted in the urine while the remainder is excreted in the stool. Zinc that is absorbed or injected is excreted in the pancreatic juice with very little appearing in the bile.[25]

Table III reveals considerable variation in reported ranges and average plasma values for zinc. Red cell values are more consistent. Our mean red cell value is 3.3 times the mean plasma value, whereas results reported by other investigators show much greater disparity. Our results are scattered uniformly but within a narrower range for plasma than for red cells. Plasma values were low in the second and third decades while a sharp rise was found in the 30 to 40 age group.

SUMMARY

One hundred and nine samples of plasma and 106 samples of red blood cells from sixty-one normal subjects were analyzed by a spectrochemical method for magnesium, chromium, nickel, copper and zinc. The ranges and mean plasma levels of these elements were found to be: magnesium 12.5 to 36.0, 20.4; chromium 0.009 to 0.055, 0.027; nickel 0.00 to 0.27, 0.060; copper 0.50 to 1.93, 1.03; zinc 0.49 to 7.70, 3.01 p.p.m. The ranges and mean red cell levels were found to be: magnesium 26.0 to 131.0, 74.3; chromium 0.005 to 0.054, 0.021; nickel 0.00 to 0.31, 0.053; copper 0.28 to 2.8, 0.82; zinc 3.6 to 25.4, 10.0 p.p.m. Statistical analysis of the data showed no significant differences attributable to sex and race. The mean plasma values for zinc were lower in the second and third decades than in the fourth decade. The mean red blood cell values for nickel in the second decade and copper in the fifth decade were found to be lower than average.

ACKNOWLEDGMENT

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Trace Metals in Human Plasma and Red Blood Cells

A Study of Magnesium, Chromium, Nickel, Copper and Zinc
II. Observations of Patients with Some Hematologic Diseases

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IN PART I OF this study, plasma and red blood cell values for magnesium, chromium, nickel, copper and zinc in normal persons are reported. The observations made of a group of patients with hematologic diseases (see Tables I and II) are reported herein.

Patients were chosen for study from the wards and Hematology Clinic of the University of Virginia Hospital. Only patients with unequivocal diagnoses were selected, and whenever possible blood samples were taken before and after treatment. The presence of complications and concurrent diseases resulted in exclusion of several patients because of the possible introduction of additional variables. The spectrochemical method of analysis was employed throughout.

MAGNESIUM

It now seems established that alterations in the content of magnesium in the body are capable of producing clinical manifestations. Since magnesium, like potassium, is chiefly an intracellular ion, the serum level is a poor measure of total deficit or excess. Doubtlessly this factor has delayed recognition of the association of magnesium with certain clinical states. Smith and Hammarsten have demonstrated this by a recent study. Of twelve patients with delirium tremens, the levels of magnesium in plasma were below the normal range in only seven, whereas values for the red blood cells were decreased in all of the patients.[1,2] Previous studies of patients with this condition have dealt only with serum values,[3,4] and have been judged inconclusive.[5] In delirium tremens the administration of magnesium is generally followed, after some delay, by improvement.[1-3]

Spontaneous normocalcemic, hypomagnesemic tetany has been reported.[6] Other conditions in which magnesium depletion may be implicated are heart failure treated with ammonium chloride and mercurial diuretics, chronic nephritis,[7] treated diabetic acidosis,[8] replacement of large fluid losses (e.g., by intubation) with fluids poor in magnesium,[2,9] and severe burns.[10] Osteolytic bone lesions with hypercalcemia and hypercalciuria,[2] hyperparathyroidism,[11,12] and primary hyperaldosteronism[13,14] have been associated with very low levels of magnesium in the serum. Reports of changes in uncomplicated portal

TABLE I

Plasma Values for Patients with Some Hematologic Diseases*

Disease	No. of Samples and Condition	Magnesium	Chromium	Nickel	Copper	Zinc
None	109 (normal)	20.4 (12.5–36.0)	0.027 (0.009–0.055)	0.060 (0–0.27)	1.03 (0.48–1.93)	3.01 (0.49–7.70)
Aplastic anemia	0 (untreated)	...	...	...	...	...
	3 (treated)	17.5 (16.3–18.1)	0.022 (0.022–0.023)	0.013 (0–0.040)	1.17 (0.86–1.71)	2.27 (1.30–3.00)
Myelofibrosis	0 (untreated)	...	...	...	...	...
	3 (treated)	21.8 (17.3–23.2)	0.024 (0.020–0.033)	0.027 (0–0.040)	0.99 (0.87–1.09)	2.50 (1.40–3.10)
Acquired hemolytic anemia	1 (untreated)	20.7	0.027	0.040	0.68	2.50
	4 (treated)	17.0 (14.3–21.7)	0.021 (0.020–0.024)	0.017 (0–0.070)	1.16 (0.64–2.30)	2.65 (1.60–4.20)
Sickle cell anemia	0 (untreated)	...	...	...	...	...
	3 (treated)	19.2 (13.3–28.5)	0.023 (0.021–0.028)	0.043 (0–0.130)	1.67 (0.95–2.20)	2.71 (1.70–4.20)
Pernicious anemia	7 (untreated)	18.6 (12.0–26.9)	0.025 (0.012–0.041)	0.053 (0–0.10)	1.21 (0.90–1.60)	1.19 (0.64–2.00)
	15 (treated)	17.6 (14.2–22.2)	0.030 (0.019–0.058)	0.051 (0–0.130)	1.02 (0.50–1.55)	3.88 (2.40–7.80)
Iron deficiency anemia	6 (untreated)	20.4 (16.4–22.8)	0.029 (0.020–0.028)	0.017 (0–0.100)	1.42 (0.82–2.04)	3.60 (2.25–4.50)
	1 (treated)	20.9	0.032	0	0.96	6.10
Polycythemia vera	3 (untreated)	21.2 (14.4–30.0)	0.043 (0.027–0.072)	0.110 (0–0.230)	1.67 (0.77–3.20)	3.90 (3.00–5.10)
	9 (treated)	18.6 (12.7–22.6)	0.029 (0.015–0.037)	0.044 (0–0.120)	1.24 (0.54–2.10)	3.80 (1.80–6.10)
Acute leukemia	3 (untreated)	21.2 (16.4–28.7)	0.026 (0.024–0.029)	0.013 (0–0.040)	1.56 (0.67–2.06)	4.03 (3.55–4.65)
	6 (treated)	22.2 (16.4–25.8)	0.023 (0.011–0.030)	0.013 (0–0.040)	1.60 (0.94–2.30)	2.89 (1.57–4.90)
Chronic myelogenous leukemia	1 (untreated)	18.6	0.016	0.10	0.88	1.40
	4 (treated)	17.7 (13.1–24.1)	0.025 (0.020–0.035)	0	0.71 (0.51–0.87)	2.44 (1.50–3.80)
Chronic lymphocytic leukemia	2 (untreated)	19.7 (19.3–20.0)	0.029 (0.023–0.035)	0	1.15 (1.06–1.23)	3.90 (3.20–4.60)
	4 (treated)	23.4 (15.8–33.3)	0.027 (0.020–0.034)	0.042 (0–0.130)	1.49 (1.19–1.95)	2.98 (2.20–3.50)
Hodgkins disease	1 (untreated)	11.5	0.018	0	1.34	1.60
	11 (treated)	18.2 (12.4–28.5)	0.027 (0.018–0.039)	0.027 (0–0.150)	1.48 (0.72–4.50)	3.35 (1.50–7.10)
Lymphosarcoma	2 (untreated)	20.3 (16.8–23.8)	0.022 (0.021–0.023)	0.020 (0–0.040)	1.32 (1.14–1.51)	2.38 (1.75–3.00)
	14 (treated)	19.0 (13.5–26.0)	0.028 (0.019–0.043)	0.024 (0–0.180)	1.45 (0.60–2.90)	3.20 (1.50–6.00)
Multiple myeloma	5 (untreated)	21.8 (15.9–29.0)	0.023 (0.017–0.027)	0.054 (0–0.130)	1.19 (0.81–1.87)	2.62 (0.72–4.40)
	3 (treated)	19.4 (15.1–27.0)	0.024 (0.022–0.026)	0.013 (0–0.040)	0.91 (0.77–1.00)	3.73 (3.10–4.20)

* Mean values and ranges are given in p.p.m. (μg. per gm.). Ranges are in parentheses.

cirrhosis and diabetes mellitus are conflicting.[2,4,15]

Hypermagnesemia has long been associated with renal failure, although it may not be observed even with severe nitrogen retention. Smith and Hammarsten found the magnesium levels in the plasma elevated in only ten of fourteen patients with uremia and depression of the central nervous system, but the red blood cell value was high in all subjects, again emphasizing the importance of the intracellular level.[1,2] Hamburger found the levels of

TABLE II
Red Blood Cell Values for Patients with Some Hematologic Diseases*

Disease	No. of Samples and Condition	Magnesium	Chromium	Nickel	Copper	Zinc
None	106 (normal)	74.3 (26.0–131.0)	0.021 (0.005–0.054)	0.053 (0–0.31)	0.82 (0.28–2.80)	10.0 (3.80–25.40)
Aplastic anemia	0 (untreated)	...	...	...	...	...
	3 (treated)	72.0 (57.7–80.6)	0.021 (0.015–0.026)	0.053 (0–0.080)	0.68 (0.49–0.84)	9.20 (6.20–13.20)
Myelo-fibrosis	0 (untreated)	...	...	...	...	...
	3 (treated)	143.0 (117.0–181.0)	0.021 (0.018–0.025)	0.080 (0.055–0.115)	0.68 (0.55–0.87)	9.33 (8.00–11.70)
Acquired hemolytic anemia	0 (untreated)	...	...	...	...	...
	4 (treated)	95.3 (73.3–136.7)	0.024 (0.016–0.035)	0.017 (0–0.030)	0.51 (0.50–0.72)	9.43 (5.90–12.80)
Sickle cell anemia	0 (untreated)	...	...	...	...	...
	2 (treated)	62.2 (53.2–71.3)	0.036 (0.032–0.039)	0.080 (0.020–0.140)	0.90 (0.74–1.06)	17.70 (15.90–19.50)
Pernicious anemia	6 (untreated)	90.9 (52.5–151.0)	0.022 (0.017–0.033)	0.070 (0–0.210)	0.81 (0.54–1.06)	8.23 (4.80–10.50)
	14 (treated)	86.3 (48.8–146.1)	0.026 (0.015–0.041)	0.032 (0–0.100)	0.87 (0.32–1.43)	11.08 (4.70–20.60)
Iron deficiency anemia	6 (untreated)	80.7 (50.7–133.2)	0.026 (0.012–0.052)	0.015 (0–0.050)	0.73 (0.64–0.81)	11.70 (8.00–15.10)
	1 (treated)	57.6	0.025	0.020	0.52	14.80
Polycy-themia vera	3 (untreated)	59.0 (32.5–80.1)	0.039 (0.024–0.059)	0.085 (0.054–0.110)	0.73 (0.38–1.10)	10.00 (6.10–14.70)
	9 (treated)	79.7 (32.1–137.0)	0.023 (0.013–0.047)	0.059 (0–0.180)	0.98 (0.61–1.87)	10.40 (6.30–20.20)
Acute leukemia	3 (untreated)	104.3 (87.7–127.0)	0.022 (0.015–0.031)	0.033 (0.020–0.060)	0.78 (0.34–1.04)	13.00 (10.10–71.20)
	6 (treated)	106.0 (81.0–135.0)	0.029 (0.023–0.044)	0.037 (0–0.077)	0.62 (0.48–0.94)	13.00 (7.20–18.70)
Chronic myelo-genous leukemia	1 (untreated)	91.0	0.026	0.130	0.22	11.50
	4 (treated)	77.6 (70.8–95.5)	0.023 (0.014–0.035)	0.049 (0–0.110)	0.63 (0.51–0.83)	10.69 (8.20–15.60)
Chronic lympho-cytic leukemia	2 (untreated)	62.5 (33.1–91.9)	0.033 (0.027–0.040)	0.075 (0.020–0.130)	0.83 (0.80–0.85)	15.20 (12.00–18.50)
	4 (treated)	102.5 (57.2–125.2)	0.018 (0.013–0.024)	0.032 (0–0.075)	1.09 (0.70–1.80)	9.80 (6.90–13.80)
Hodgkins disease	1 (untreated)	77.2	0.022	0.020	0.79	9.70
	10 (treated)	82.1 (40.3–141.0)	0.024 (0.017–0.031)	0.028 (0–0.085)	1.03 (0.40–2.50)	9.84 (6.20–14.20)
Lympho-sarcoma	2 (untreated)	83.8 (76.9–90.8)	0.015 (0.014–0.015)	0.040 (0.020–0.060)	0.90 (0.66–1.14)	6.45 (5.30–7.60)
	14 (treated)	70.2 (31.2–129.8)	0.025 (0.013–0.035)	0.043 (0–0.140)	1.00 (0.36–2.20)	10.50 (3.20–15.20)
Multiple myeloma	5 (untreated)	82.9 (38.8–121.0)	0.025 (0.016–0.020)	0.067 (0.020–0.120)	1.11 (0.63–1.80)	10.10 (5.10–12.30)
	2 (treated)	55.1 (37.2–72.9)	0.034 (0.034–0.035)	0.075 (0.020–0.130)	1.08 (0.54–1.60)	14.35 (14.30–14.40)

* Mean values and ranges are given in p.p.m. Ranges are in parentheses.

magnesium in the serum to be constantly increased in patients in the anuric state and to correlate well with clouding of the consciousness and prolongation of the Q-T interval of of the electrocardiogram.[16] The levels of magnesium in the serum were found to be elevated in patients with adrenal insufficiency.[17]

For a detailed discussion of disturbances in magnesium metabolism the complete and critical review by Wacker and Vallee is recommended.[5]

We have found no reports of magnesium

abnormalities associated with hematologic disorders.

In none of the diseases studied did we find abnormal levels of magnesium in plasma, except for one patient with untreated Hodgkin's disease whose plasma value was low.   (Table I.)

The levels of magnesium in the red blood cells (Table II) were above the normal range in all three patients with myelofibrosis.   In patients with acute leukemia, marked elevations were also observed.   One of these patients had acute lymphoblastic leukemia.   The value for his red blood cells before treatment was high (127 p.p.m.) but returned to normal after one month of treatment with 6-mercaptopurine. Three of four samples from patients with treated chronic lymphocytic leukemia yielded extremely high values.   The fourth sample was from a patient who was treated ten years before by radiation with x-rays to the spleen.   The value for his red blood cells was normal as in patients with untreated chronic lymphocytic leukemia.

CHROMIUM

Strong evidence exists that chromium is a cancerigenic agent.   This is suggested by experiments with rats[18] as well as by epidemiologic studies of workers exposed to high concentrations of chromium compounds in industry.[19-21]   It is of particular interest that this malignant influence seems to be chiefly confined in man to the development of bronchogenic carcinoma.[19,20,22-25]   The reason for this is unknown.

Ulceration of the skin and nasal septum and a variety of inflammatory conditions of the respiratory tract are common in persons exposed to chromium.[26-28]   Contact dermatitis from shoe leather is also an occasional problem.[28] Magnus suggests that chromium enters the epidermis in hexavalent form (which may have direct cytotoxic effects[23]), then is reduced to the trivalent cation which is able to bind protein and thereby induce sensitivity.[29]   However, Morris believes that basic chromic sulfate, containing the trivalent cation, is the cause of dermatitis from shoe leather and that in this form chromium (III) may enter the skin directly, being conveyed by perspiration.[28]

We have encountered no reports of studies concerning chromium in the blood or of chromate exposure in patients with hematologic diseases.

In our investigation, values for chromium were found to be consistently normal in both plasma (Table I) and red blood cells (Table II) in all of the diseases studied.

NICKEL

It appears probable that nickel is capable of causing carcinoma of the respiratory tract. Doll[30] estimates the risk of death from cancer of the lung to be five times the normal rate, and from cancer of the nose, 150 times the normal rate, among workers in the nickel refineries in South Wales who were employed prior to 1924.   Kincaid et al. believe the responsible agent to be nickel carbonyl.[31]

Nickel carbonyl may cause severe acute intoxication.   Immediately after exposure, headache, giddiness, "tightness" in the chest, nausea and weakness may occur, or there may be an asymptomatic period of several days followed by a delayed reaction.   This is marked by severe retrosternal pain, dyspnea, weakness and occasional gastrointestinal or genitourinary symptoms.   Delirium and convulsions may occur terminally.[32]   In fatal cases, death appears to result from severe damage to lung tissue.[33]

The administration of sodium diethyldithiocarbamate to the patient is followed by a marked increase in urinary excretion of nickel which gradually returns to normal, and the patient clinically recovers within two to three weeks.[32]   This agent seems to be highly effective therapeutically and probably acts by forming a non-toxic chelate with nickel, which is then rapidly excreted.[34]

The extreme toxicity of nickel carbonyl is recognized by the American Conference of Governmental Industrial Hygienists, which sets the maximum concentration allowable for workmen for an eight hour day at 0.001 p.p.m. in air.[35]

Metallic nickel is important as a cause of contact dermatitis[36] and may cause cross sensitivity with copper.[27]   It also has the ability, peculiar among contact allergens, to cause a

secondary eruption in areas other than the site of contact.[27,36] Wells believes that sweat may mobilize nickel from metallic contactants, and has demonstrated a peculiar affinity of keratin for this metal. It is his opinion that penetration into the deeper layers of epidermis occurs only at sweat duct and hair follicle openings.[37] Magnus suggests that linkage with soluble proteins may occur in the Malpighian layer.[29]

There are no reports relating nickel to diseases of the blood.

We found the nickel values to be highly variable in the plasma and red blood cells of patients with the diseases which we studied (Tables I and II). We believe that no valid conclusions can be drawn from the data obtained.

COPPER

Deficiency of naturally occurring copper has been observed for some time in ruminants grazing where the forage is poor in copper. Cachexia and poor development of the coat result. In lambs, spastic paralysis has been observed with cerebral demyelination and degeneration of motor tracts in the spinal cord. Deficiency of copper in ruminants has also been found where normal or increased amounts of the metal occur in the herbage; this is presumably due to its presence in an unavailable form.[38]

The occurrence of disease in man due to copper deficiency has not been demonstrated. However, there are many conditions which have been associated with alterations in the levels of copper in the blood. Hypocupremia has been found in patients with the nephrotic syndrome; this is presumably due to the loss of ceruloplasmin through the urine.[39] Hypocupremia has been described as part of a syndrome, occurring in infants, which is characterized by edema, anemia, hypoproteinemia, low levels of iron in the serum and a limited iron-binding capacity. This syndrome has been attributed to a transient unexplained hypercatabolism of protein.[40] A similar condition in infants, thought to be due to copper depletion, has also been reported.[41] Hypocupremia occurs in children with kwashiorkor,[42] sprue[41] and in some infants with iron deficiency anemia.[40] In persons with Wilson's disease, ceruloplasmin is decreased or absent,

probably because of a failure in synthesis which is thought to account for the hypocupremia.[43]

Hypocupremia occurs in persons in normal pregnancy and in those with one of a large number of pathologic conditions.[44,45] Treatment with ACTH of patients with acute leukemia resulted in a decline of the levels of copper in plasma which, upon relapse, again became high.[44] The administration of estrogens to thirteen women with gynecologic complaints was followed by an increase in the levels of copper in the serum.[46]

High levels of copper in tissue occur in patients with Wilson's disease in spite of hypocupremia.[48] Koch et al. found normal or only slightly elevated values in most tissues studied which were obtained from eight patients with lymphomas, but the levels in the plasma were high in all of these patients.[47] Toxic and nontoxic goiters have been found to contain increased amounts of copper.[48] In persons with cirrhosis with cholangitis, the liver was found to have a markedly elevated content of copper; this was attributed to failure of excretion by the liver due to biliary obstruction.[49]

The levels of copper in the red blood cells were found to be normal in most of the patients with the conditions studied by Lahey et al. They found the levels to be elevated in only five of seventeen patients with iron deficiency anemia;[44] whereas, Pagliardi et al. observed increased values in all of sixteen patients; these returned to normal upon treatment with iron or blood transfusions. The levels of copper in the red blood cells have been reported to be elevated in patients with a variety of other conditions.[50] Low values for the red blood cells have been determined in persons with carcinomas and hyperthyroidism.[44]

In our study, levels of copper in plasma were found to be elevated in patients with several conditions. Nineteen of twenty-eight samples from patients with Hodgkin's disease and lymphosarcoma showed moderate to marked elevations. These two groups yielded average values that were 50 per cent higher than normal. Only two patients with acute leukemia had extremely high levels and these account for our high average value. One of these returned to normal following treatment of the patient

with 6-mercaptopurine. Half the samples from patients with pernicious anemia showed moderately high levels of copper in the plasma; no differences were attributable to treatment with vitamin B_{12}. High levels of copper in the plasma from persons with polycythemia vera were found in only a third of the samples.

Contrary to previous reports,[44,50] we found no elevation of the levels of copper in the red blood cells of six patients with iron deficiency anemia. Samples from the one patient, who was re-examined after treatment with ferrous sulfate at which time his blood count was normal, showed a marked decline in the level of copper in his red blood cells. No consistent abnormality in the amount of copper in the red blood cells was observed.

ZINC

The occurrence of disease due to primary zinc deficiency has not been established in man, however, it is recognized in swine as the cause of parakeratosis. A high intake of calcium aggravates this deficiency.[51] A few balance studies of man have been carried out, but most of the conditions studied are characterized by lowered values for the serum and values for the red blood cells which vary directly with the hematocrit and hemoglobin concentration.[52] In women who had recently delivered, Vikbladh found the levels of zinc in the serum to be slightly lower than normal, but in their newborn infants the values were higher than normal. The values for erythrocytic zinc were slightly elevated in the mothers but markedly reduced in the infants.[53]

Vallee et al. have found a marked decrease in the levels of zinc in the serum of patients with Laennec's cirrhosis. Tissue from the livers of these patients contained subnormal amounts of zinc, but large quantities were excreted in the urine, except in that of one patient who was terminally ill. It is of especial interest that the administration of zinc tended to restore normal excretory patterns.[54,55] These findings suggest zinc depletion but its relation to the pathogenesis of cirrhosis is not yet clear.

Reduction of the levels of zinc in the serum occurs in patients with myocardial infarction and has an approximately inverse relationship to the levels of activity of serum lactic and malic dehydrogenases. It is suggested that this reduction in zinc removes an inhibition, allowing increased activity of these enzymes. This same relationship is present in persons with cirrhosis and pernicious anemia.[56]

Vikbladh observed a decrease in the levels of zinc in the serum of patients with several acute febrile conditions. Statistical analysis showed a negative correlation between the serum zinc level and the amount of fever of the patient. Low values for zinc in the serum of patients with malignant tumors, hepatogenic jaundice, chronic polyarthritis and chronic nephritis were also found. The levels of zinc in the serum were normal for persons with afebrile rheumatic fever, acute nephritis, peptic ulcer and diabetes.[53]

Several persons with diseases of the blood and the blood-forming organs have been studied with respect to the content of zinc in the serum. These values were normal for patients with anemia following gastrointestinal bleeding with the exception of those with bleeding from carcinoma of the stomach in which case the levels appeared to be reduced. Eight patients with iron deficiency anemia had normal levels. However, the levels of zinc in the serum were low for all of the fourteen patients with untreated pernicious anemia; they returned to normal levels with therapy through the administration of liver.[53] Vallee and Gibson reported the zinc content of the red cells to be high in persons with pernicious anemia, and to subsequently diminish with treatment; these values were obtained by calculations based on measurements of the levels in plasma and whole blood.[57] Vikbladh observed low levels of zinc in the serum of patients with leukemia, but equivocal results were obtained from those with multiple myelomata and aplastic and hemolytic anemias.[53] The leukocyte content of zinc was found to be about 10 per cent of normal in persons with myelogenous and lymphatic leukemia.[57]

The content of zinc in tissue has been studied best in persons with diseases of the prostate. Low values were obtained for those with prostatic hyperplasia and prostatitis and even lower values were found in tissues from

cancerous glands.[58,59] Therapy with stilbestrol lowered the values slightly more. Koch et al. found increased amounts of zinc in the thyroids of patients with papillary adenocarcinomas[60] and normal or slightly elevated values in most tissues of patients with lymphomas.[47]

Intoxication from zinc is rarely encountered. Grant-Frost and Underwood demonstrated that zinc is capable of inducing severe copper deficiency in the rat.[61] Ingestion of zinc compounds has been implicated in instances of acute[62] and chronic[63] poisoning. Zinc chloride is highly toxic on contact with tissue and may cause dermatitis.[64] Inhalation of zinc chloride smoke has resulted in extensive damage to the respiratory tract with a subsequent high rate of mortality.[65] Inhalation of zinc oxide fumes may cause metal-fume fever, a benign systemic disease.[66] Ethylenediamine tetra-acetate produces a diuresis of zinc[67] and would probably be useful in the treatment of persons with chronic zinc intoxication.

Our data indicate consistently low values for zinc in the plasma from patients with untreated pernicious anemia (Table I) and are in agreement with those of previous studies of serum from patients with this condition.[53,57] This suggests that the clotting process does not remove zinc. After treatment of the patients with vitamin B_{12}, the level in the plasma were found to be normal or elevated. Examination of data from individual patients suggests a gradual rise in the level of zinc, following treatment, to a supernormal peak at two to three months and a subsequent decline to normal range. From all three patients with untreated acute leukemia, extremely high values for zinc in the plasma were obtained.

In contrast to the finding of Vallee and Gibson,[57] we obtained normal values for zinc in the red blood cells of all of the four patients with untreated pernicious anemia. Following specific therapy, a slight but inconstant rise was observed. We found no consistent abnormalities concerning the content of zinc in the red blood cells (Table II).

COMMENTS

Except for those patients with pernicious anemia, no differences clearly due to treatment were observed in the patients with these hematologic diseases. Therapy included phlebotomy for those with polycythemia vera, 6-mercaptopurine and prednisone for patients with acute leukemia, alkylating agents and irradiation with x-rays for those with lymphomatous diseases, urethane for persons with multiple myeloma and blood transfusions for patients in most of the groups. One patient with aplastic anemia, who had received a total of sixty-nine blood transfusions, had normal values.

In view of the experiment of Grant-Frost and Underwood,[61] our data were examined to see if a reciprocal relationship exists between copper and zinc. The plasma values showed such a relationship in thirty-six of the 111 samples. In this group, the values for either copper or zinc were definitely above the normal mean value while the others were definitely below. In fifty-eight samples, the levels of either copper or zinc or both were close to the mean. In only seventeen patients, were the values for both metals increased or decreased. This reciprocal relationship was more pronounced in the red blood cells as it was observed in forty-two of the 106 samples. In fifty-seven samples, values for either or both copper and zinc were close to the normal mean, and in only seven persons were the levels of both metals increased or decreased.

SUMMARY

Samples of plasma and red blood cells from seventy-eight adult patients with hematologic diseases were analyzed spectrochemically for magnesium, chromium, nickel, copper and zinc. The literature was briefly reviewed and our data compared with those provided in available reports. Our results are tabulated and discussed.

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A Repository Vitamin B_{12} Preparation: Cyanocobalamin Zinc Tannate

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INTRAMUSCULARLY administered crystalline cyanocobalamin is rapidly excreted into the urine, and the larger the dose, the smaller is the percentage retained in the body.[1] It would appear that regardless of the amount of vitamin B_{12} injected, the body has a limited capacity to bind and retain the vitamin,[2] and the free or unbound fraction of an injected dose of cyanocobalamin is eliminated rapidly into the urine.

It is probable that the serum concentration of vitamin B_{12} in man falls to deficiency levels (less than 100 $\mu\mu g./ml.$) only when the tissue reservoirs, chiefly the liver, have been almost depleted.[3] Indeed, in pernicious anemia the liver has been found to contain no vitamin B_{12}.[4] By calculation, the normal serum concentration (350 to 560 $\mu\mu g./ml.$) represents only 1 or 2 $\mu g.$ of vitamin B_{12} in the total circulation,[3] and hence, the injection of a very small amount of cyanocobalamin can restore circulating concentrations to normal. The mere restoration of the circulating quantities of the vitamin does not represent the true objective of therapy of a patient with deficiency and exhausted tissue reservoirs.

Regardless of the size of the intramuscularly administered dose of crystalline cyanocobalamin, only that fraction which is retained in the body can go toward repletion of body stores. It follows that prolongation of the time during which cyanocobalamin is circulated within the body favors retention. The relatively insoluble cyanocobalamin zinc tannate§ complex has the potentials of slow release from the site of injection and prolonged periods of time during which body proteins can bind and retain vitamin B_{12}.

The present study was directed toward discovering the extent to which cyanocobalamin zinc tannate merits the designation of a repository form of vitamin B_{12}.

MATERIALS AND METHODS

The lyophilized, limitedly soluble cyanocobalamin zinc tannate complex is supplied in multidose vials, and when restored with the diluent, provides an aqueous suspension containing 500 $\mu g.$ cyanocobalamin, 1.2 mg. zinc and 2.6 mg. tannic acid per ml.[5] There was minimal pain following its intramuscular injection in quantities of either 1 or 2 ml.

A series of twenty-five persons was studied, all of whom were healthy ambulatory adults, varying in age from nineteen to fifty-three years, and in weight from 108 to 230 pounds. The group consisted of twenty-one women and four men. Eleven persons received intramuscular injections of 500 $\mu g.$ and fourteen were injected with 1,000 $\mu g.$ Pretreatment blood samples were drawn, and after treatment, samples were obtained from some patients at one, three and six hours and then at four, eight, twelve, sixteen, nineteen, twenty-four and twenty-seven days thereafter, and from others at slightly different times, three, seven, eleven, fifteen, eighteen and twenty-one days. Blood samples were allowed to clot in the refrigerator overnight, the serum har-

TABLE I

Serum B$_{12}$ Concentrations ($\mu\mu$g.) Following a Single Intramuscular Injection of Vitamin B$_{12}$ Tannate* (500 μg.)

Patient	Sex	Age	Weight (lb.)	Premedication	Time After Dose									
					Day 1			Day 4	Day 8	Day 12	Day 16	Day 19	Day 24	Day 27
					1 hr.	3 hr.	6 hr.							
B	F	49	245	520	2,300	2,400	2,200	900	1,000	490	490	460	460	340
F	F	33	165	570	1,200	1,400	2,000	3,800	2,100	...	1,200	780	610	750
K	F	53	162	190	1,200	1,100	800	375	100	250	290	230	300	260
P	F	49	134	550	1,700	2,600	2,500	1,800	3,000	2,700	1,640	1,260	960	1,090
SC	F	38	168	580	2,800	2,300	1,900	2,600	2,100	420	...	...	410	490
SH	F	25	135	330	450	700	150	500	700	730	630	520	410	330
Average				457	1,608	1,750	1,592	1,663	1,500	918	850	650	525	543

Patient	Sex	Age	Weight (lb.)	Premedication	Time After Dose									
					Day 1			Day 3	Day 7	Day 11	Day 16	Day 19	Day 22	Day 25
					1 hr.	3 hr.	6 hr.							
WMM	F	27	110	640	2,700	1,750	1,000	1,050	800	920	980	640	560	720
HW	M	31	230	200	900	750	820	700	560	780	440	410	360	370
BD	F	30	138	140	1,200	1,000	1,500	1,600	1,800	2,075	1,700	1,130	1,200	1,200
MMcL	F	37	110	<100	500	500	600	900	600	240	200	...	...	...†
RG	M	27	150	240	1,000	4,100	1,500	2,700	1,000	920	1,260	...	800	400
Average				264	1,260	1,620	1,150	1,562	1,300	987	916	727	730	672

* Depinar Lot No. U202.
† On the thirty-first day after injection serum concentration of vitamin B$_{12}$ was 140 $\mu\mu$g.

vested into 10 ml. sterile serum vials, and stored at minus 20°c. until assayed.

Serum concentrations were estimated by microbiologic assay using the Lactobacillus leischmania method.[6] All specimens from any particular patient were assayed during the same test, so that assay conditions were constant for that patient.

RESULTS

The serum vitamin B$_{12}$ concentrations following single intramuscular injections of 500 μg. of cyanocobalamin zinc tannate are presented in Table I, and those following single injections of 1,000 μg. of the same preparation are presented in Table II. The different time schedules for sampling are obvious.

It should be noted that patient M. McL., female, age thirty-seven, and L. W., female, age forty-two, both showed pretreatment serum B$_{12}$ concentrations less than 100 $\mu\mu$g./ ml. No reason could be found for these abnormally low values; blood counts were normal, free gastric acidity was demonstrated,

no neurologic symptoms were found, and there was no familial history of pernicious anemia. Further, observations on patient M. McL. are presented in Table III.

COMMENTS

It has been well established that cyanocobalamin is preponderantly transported and bound by a$_1$ and a$_2$ globulins,[2,7,8] and free or unbound cyanocobalamin is rapidly excreted by the kidneys. Under normal conditions of health, it may be postulated that the serum concentrations of vitamin B$_{12}$ will be the resultant of (1) the small daily quantities of the vitamins derived from the diet that are absorbed through the gastrointestinal mucosa; (2) the modest daily metabolic requirement for the vitamin; and (3) the capacity of body tissue to bind and retain cyanocobalamin. A delicate balance is struck between these various factors under normal circumstances. The estimated quantities of vitamin B$_{12}$ in the average diet[9] are 0.2 to 3.5 μg.; the daily requirement[9] for the vitamin, 0.1 to 1

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TABLE II
Serum B_{12} Concentrations ($\mu\mu$g.) Following a Single Intramuscular Injection of Vitamin B_{12} Tannate* (1,000 μg.)

Patient	Sex	Age	Weight	Premed-ication	Day 1			Day 3	Day 7	Day 11	Day 16	Day 19	Day 22	Day 25
					1 hr.	3 hr.	6 hr.							
JW	M	40	167	200	2,000	1,600	1,700	1,800	800	600	500	450	460	400
EQ	M	37	175	440	2,600	3,000	2,100	2,200	788	560	800	400	480	460
RL	F	30	130	200	2,600	2,300	1,650	1,350	1,000	...	520	660	500	470
LW	F	42	108	<100	400	3,000	1,500	1,100	1,300	880	580	400	360	460
EB	F	43	140	800	1,200	1,000	2,900	2,500	1,100	500	660	560	550	420
DS	F	39	135	630	3,200	2,800	2,250	2,000	2,500	2,200	3,150	2,000	1,300	580
Average				395	2,000	2,283	2,017	1,825	1,248	948	1,035	745	608	465

Patient	Sex	Age	Weight	Premed-ication	Day 1			Day 4	Day 8	Day 12	Day 16	Day 19	Day 24	Day 27
					1 hr.	3 hr.	6 hr.							
BL	F	19	154	300	1,500	1,000	1,500	2,100	1,900	...	1,460	600	690	200
CO	F	39	150	200	1,700	2,900	2,600	1,400	1,400	960	860	760	610	...
CO	F	41	169	310	2,300	2,600	1,900	1,900	2,100	680	650	520	570	440
DE	F	36	138	250	3,300	3,100	1,100	600	950	250	230	280	390	100
H	F	42	136	...	2,400	1,600	...	1,200	350	340	360	300	280	180
SM	F	50	180	400	2,100	3,000	2,000	2,700	3,200	1,640	1,300	1,040	860	960
ST	F	33	150	310	1,400	4,200	1,700	800	1,200	1,130	980	1,620	1,010	1,030
R	F	20	112	...	...	1,800	1,600	850	...	680	600	380	270	210
Average				295	2,100	2,525	1,771	1,444	1,588	811	805	688	585	448

* Depinar Lot No. U202.

TABLE III
Serum B_{12} Concentrations Observed during Treatment with Cyanocobalamin Zinc Tannate* over a Period of 191 Days

Date	Amount (μg.)	0 (Control)	3	4	5	7	8	10	11	14	16	30	31	54
8/24/59	500	<100	900	...	...	600	...	...	240	... (11/30/59)	200	...	140	<100
11/16/59	1,000	...	...	950	...	...	950	...	...	530 (12/14/59)	...	...	...	...
11/30/59	1,000	...	...	2,080	...	1,760	...	...	900	660	...	...	...	...
12/14/59	1,000	...	...	...	2.200	...	...	(12/28/60) 2,800	...	...	...	...	...	...
12/28/59	1,000	...	...	...	...	...	...	...	...	...	...	380	...	...
2/1/60	1,000	...	...	...	...	...	...	...	...	...	...	840	...	...

* Depinar Lot No. U202.

μg. and the amount of circulating vitamin, 1 to 2 μg., which reflects the normal serum concentration of 350 or 560 $\mu\mu$g./ml.[3,10] In a healthy person the liver is always a storehouse containing 1,000 to 2,000 μg. of B_{12}[4,11] so that if all extraneous sources of the vitamin are cut off, it will be three to six years before a deficiency state is established.[11]

In a group of our patients followed up over a period of years, it has been established that each person has a more or less constant serum concentration of B_{12}, determined by his own physiology. Various schedules of therapy are capable of temporarily altering the levels at which vitamin B_{12} circulates; however, shortly after the discontinuance of therapy, the

patient re-establishes his "normal." These observations clearly suggest a defined limitation of the patient to bind and retain cyanocobalamin. Interestingly enough, the two conditions in which serum concentrations of vitamin B_{12} have most consistently been shown to be elevated, acute or chronic myelogenous leukemia[2,8] and hepatocellular jaundice,[12] are both conditions in which globulins are abnormal

There is a sharp limitation of time during which the body tissues and blood proteins have the opportunity of binding the vitamin when cyanocobalamin is administered by injection. One must assume either that (1) during the period of six to eight hours following the injection of crystalline vitamin B_{12}, all unsaturated body proteins bind the vitamin to their fullest capacity, or (2) the time during which excess quantities of vitamin circulate following an intramuscular injection is inadequate for the full saturation of proteins capable of binding B_{12}.

Extensive work[1] has shown that as intramuscular injections exceed 30 to 40 μg., the amount of the vitamin excreted into the urine progressively increases, certainly implying a limited capacity to bind and retain the vitamin. The question remains, however, as to whether the limited time during which the proteins have an opportunity to bind the circulating quantities of B_{12} may be too short for optimal or complete saturation to occur. The interesting properties of cyanocobalamin zinc tannate seem to bear upon this inquiry.

In Tables I and II are the individual data showing serum concentrations of twenty-five persons over twenty-five and twenty-seven day periods following the intramuscular injection of 500 and 1,000 μg., respectively, of cyanocobalamin zinc tannate. Variations from person to person are obvious, but there is a general and uniform trend. From the premedication control level, there is a prompt rise to peak concentrations within the first six hours following injection, and then a slow decline to pretreatment level. This decline occurred in a few patients after as short a time as eight days, but in others it had not occurred within twenty-seven days, at which time the

study was terminated. The *average* figures clearly show an elevation of serum concentration above pretreatment level for the entire duration of the study.

In previous publications, we have emphasized the inherent error of the microbiologic assay of vitamin B_{12}[13] and have joined others[14] in the statement that a 20 per cent plus or minus error should be conceded. If one applies the harshest criteria to the data in Tables I and II, the following adjustments can be made. If the *highest* average pretreatment concentration, 467, is adjusted upward by 20 per cent to 548 $\mu\mu$g./ml., and if the *lowest* average serum concentration, 805, following treatment is adjusted downward by 20 per cent to 644 $\mu\mu$g./ml., it still can be concluded that a single intramuscular injection of 500 or 1,000 μg. of cyanocobalamin zinc tannate elevates serum concentrations above pretreatment levels for at least sixteen days.

Peak serum concentrations, higher after 1,000 μg. than after 500 μg., are observed during the first three hours after injections. However, the average serum concentrations thereafter are not strikingly different. Our observation that serum concentration promptly reached its peak is at variance with previous observations that ". . .there was no large increase in serum level of vitamin B_{12} activity until the end of the first day (1,600 $\mu\mu$g./ml.),"[15] but does conform with the statement of others that "a certain portion of the vitamin B_{12} (about 10 to 15 per cent in the CZT suspension, is rapidly absorbed, as shown by the rat excretion during the first six hours after injection."[5] It should be emphasized that a "normal" serum concentration of vitamin B_{12}, 350 to 560 $\mu\mu$g./ml., represents only 1 to 2 μg. of the vitamin in the total circulation. It is clear then that the elevation of serum concentration to 1,000 or 2,000 $\mu\mu$g./ml. reflects an increase of only a few micrograms of cyanocobalamin.

In vitro studies indicate a limit to the amount of cyanocobalamin that blood proteins can bind[2] and this limit is approximately 350 to 400 $\mu\mu$g./ml. It seems reasonable to assume, therefore, that when values of serum concentration exceed this figure, those amounts in excess

of it represent free, or loosely bound, B_{12}. The method of assay employed in the present study measured only "total vitamin B_{12} activity."

There are many analogies between the dose response curves following the intramuscular injection of vitamin B_{12} and penicillin. The rapidity with which the serum concentrations decline following the intramuscular injection of crystalline vitamin B_{12} is quite similar to that observed following the intramuscular injection of sodium or potassium penicillin G. The recovery of a major portion of the injected dose, 60 to 80 per cent, within six to eight hours following injection, is also similar. Despite the use of vehicles designed to retard the absorption of penicillin, little was accomplished until a relatively insoluble salt of penicillin was prepared. The analogy between the repository forms of penicillin and cyanocobalamin zinc tannate is inescapable. Indeed, prolonged penicillemia three to four weeks following the injection of dibenzyl-ethylene diamine penicillin (DBED) is quite comparable to our findings of a two to four week elevation of serum concentrations of the vitamin.

Considering cyanocobalamin zinc tannate to be a repository vitamin B_{12} preparation, it is interesting to make certain theoretic calculations. If it is granted that a normal serum vitamin B_{12} concentration of 500 $\mu\mu$g. represents 1 to 2 μg. of the vitamin in the total circulation, and that this normal circulating value could be doubled by doubling the amount of available cyanocobalamin, it is clear that an additional 1 or 2 μg. of vitamin B_{12} could give a considerably elevated serum concentration for a variable period of time, dependent upon the rate of utilization and/or elimination. If one arbitrarily assumes that the tabularized data on serum concentrations presented in this paper justify the statement that an injection of 500 μg. of cyanocobalamin zinc tannate produced an average elevation of serum concentrations for a period of twenty-five days, one can calculate that the release of approximately 1 μg. of the vitamin per hour from the depot at the site of injection would account for the observed results. Obviously, vitamin was not released from the site of injection at any such *regular* rate. All the observations

suggest that there was a more rapid release in the first days following injection and thereafter a steadily decreasing effect upon serum concentrations.

The observations made on patient M. McL. are of particular interest (Table III). From a pretreatment level of less than 100 $\mu\mu$g./ml., regarded as the deficiency range, the patient showed a prompt peak concentration which persisted for some time following the intramuscular injection of 500 μg. of cyanocobalamin zinc tannate. At the end of thirty-one days, the pretreatment level was approximated and at some time prior to the fifty-fourth day, the patient had returned to her original status. If prolonged circulation of high concentrations of B_{12} offers a better opportunity for body proteins capable of binding B_{12} to saturate themselves, and the normal daily requirement for vitamin B_{12} is approximately 1 μg., one might have anticipated that the patient would not have returned to pretreatment level so rapidly.

Following this first injection, "normal" (560 $\mu\mu$g./ml.) or above normal serum concentrations were attained for a period of at least seven days. Following the second injection of cyanocobalamin zinc tannate of 1,000 μg., the concentrations of "normal" or above were maintained for a period of approximately fourteen days.

Without waiting a return to pretreatment levels, this patient was given several additional intramuscular injections of 1,000 μg. of repository vitamin B_{12}. From the previous experience with this patient, it is suggested that approximately thirty days were required to deplete a particular depot. Accordingly injections given at intervals shorter than thirty days should give rise to a cumulative effect. This appears to have occurred in this patient.

It is postulated that each person has a finite quantity of protein in his body capable of binding and retaining cyanocobalamin. Although a repository form of vitamin B_{12}, such as cyanocobalamin zinc tannate, can produce high circulating serum concentrations for prolonged periods of time, thus furnishing an optimal period in which the body can saturate itself, it is the person's total pool of

protein capable of binding B_{12} that constitutes the limiting factor of retention of the vitamin in the body. Amounts in excess of the ability of the body to bind, are excreted.

SUMMARY AND CONCLUSIONS

Cyanocobalamin zinc tannate, when injected intramuscularly, is released slowly from the site of its injection and can appropriately be called a depot or repository type of vitamin B_{12}. When twenty-five persons were injected intramuscularly with doses of 500 or 1,000 μg., peak serum concentrations of vitamin B_{12} were observed within three hours, and elevated concentrations persisted for periods up to twenty-seven days. A few patients showed a return of serum concentrations to pretreatment levels within eight and ten days.

Cyanocobalamin zinc tannate would appear to be a true repository vitamin B_{12} preparation and one which should make unnecessary the repeated intramuscular injections of small quantities of vitamin B_{12} for the repletion of the exhausted body stores that are observed in true vitamin B_{12} deficiency. The slow release over a period of many days of quantities of vitamin B_{12} that are greatly in excess of daily requirements, should make the treatment of "suboptimal nutritional states" that have been postulated (1) more economical, (2) more convenient and comfortable for the patient, and (3) less wasteful of personnel time in administering treatment. Perchance large quantities of circulating vitamin B_{12} do exert pharmacologic effects over and above the essential enzymatic function, then this repository form of cyanocobalamin should lend itself to the pursuit of such inquiry. It is suggested that cyanocobalamin zinc tannate may have great value in the institutional practice of medicine and in the treatment of large undernourished populations, when available skilled hands are few and the need for economy in therapy is mandatory. There would appear to be an analogy between the mass use in public health of repository penicillins and the potentials for use of this new cyanocobalamin zinc tannate.

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Some Observations on the Nutritional Status of Medical Students in the Brazilian Amazon

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No serious work has been published on the nutritional status of various population groups in the Brazilian Amazon area (an area which comprises 60 per cent of the national territory of Brazil). Within the period of 1955–1958 I had the opportunity to examine thousands of people in this area regarding their health and nutritional status. This is a report of my observations of 133 medical students in the capital city of Belem made during a study of blood pressure[1] between September 1955 and August 1956.

SUBJECTS AND METHODS

The subjects in this study represented the student body of the Medical Faculty of the University of Para in Belem, Brazil, exclusive of the last year students. All were natives of the Amazon valley, with the exception of one girl who was born in Germany. They belonged overwhelmingly to the white upper class of this area with a few mixed groups among them (16 per cent). Most of their forefathers had come from Portugal; a few from Spain, France and Germany. There were 102 men and thirty-one women with a mean age of 23.5 ± 3.4 years (men) and 22.3 ± 2.6 years (women).

Dietary Data

Seventy-six men and twenty-eight women kept a seven day individual diet record between September and November 1955 at their home after receiving careful instruction from me. Most of the students used ordinary household measures; a few, however, recorded the

amounts eaten in grams after actual weighing of the portions. A small number (15 per cent) kept a second seven day diet record in April 1956 during the winter (season of heavy rains).

After conversion of all quantities into grams the mean daily consumption per student was calculated based on raw food values as given in the only Brazilian food tables[2] available.

Physical Data

The physical data were obtained during an examination by me between September and October 1955. They were recorded on a special form and included the following information: (1) data on the family and personal history; (2) measurements of height and weight; and (3) results of the physical examination of various systems with particular attention to the skin, eyes, mouth, neck and cardiovascular system.

RESULTS

Dietary Findings

Table I gives an idea of the dietary pattern of the students. From the table it becomes apparent that bread, rice and beans were the main sources of carbohydrates, the bread being white wheat with 10 per cent manioc flour (cassava). The main source of protein was beef; main fats used were butter and vegetable oils. The men drank an average of four cups of coffee a day, whereas the women drank only about one to one and a half cups a day.

The calcium to phosphorus ratio was 1:2 in the men and 1:1.7 in the women (Table II). The protein intake in the women was significantly lower than in the men; no other differences were significant. Compared to the National Research Council's (N.R.C.) recommended dietary allowances, the dietary intake

TABLE I

Mean Weekly Consumption of Main Foods—Raw Values (gm./student)

Subjects	No.	Milk (ml.)	Bread	Beef	Chicken	Pork	Beans	Rice	Butter and Oil	Coffee (ml.)
Men	76	1,793	1,153	941	68	60	327	671	410	1,361
Women	28	1,729	806	970	106	21	144	480	354	548

TABLE II

Mean Daily Intake per Student of Various Essential Nutrients; Percentage in Relation to National Research Council Allowances[3]

Nutrient	Men (76)			Women (28)		
	Mean Daily Intake	% of N.R.C.	Range	Mean Daily Intake	% of N.R.C.	Range
Calories	2,620.0	87.3	1,194.1–4,190.8	1,990.0	86.5	812.8–3,205.7
Protein (gm.)	72.0	110.8	53.7–255.3	39.0	70.9	47.0–130.4
Calcium (gm.)	0.75	93.7	0.23–1.77	0.72	90.0	0.14–2.22
Iron (mg.)	15.0	125.0	5.91–20.79	11.3	94.2	3.74–17.89
Vitamin A (I.U.)	4.366	87.3	813–19,656	3.928	78.6	702–18,568
Thiamine (mg.)	1.9	118.9	1.01–3.39	1.3	108.3	0.50–3.23
Riboflavin (mg.)	3.2	200.0	1.31–7.36	2.6	185.7	0.72–4.32
Niacin (mg.)	23.8	148.7	12.6–45.5	17.0	141.7	8.3–43.0
Ascorbic acid (mg.)	84.9	113.2	23.9–208.1	76.1	108.7	23.4–185.3

of the male students exceeded these allowances in all instances except calories and vitamin A which were 7 to 13 per cent lower; in the women the intake of calories, protein, calcium, iron and vitamin A was 6 to 29 per cent below N.R.C. allowances. There were no major differences in the dietary intake of twelve male students and eight female students who filled a second weekly record in April 1956 for the two seasons. In April 1956 the twelve men ate slightly more than they had in September 1955, which was manifest in higher mean quotas of calories, proteins, fats, calcium and ascorbic acid. The eight women did not demonstrate the same tendency; in fact, their mean intake of proteins, fats, iron and ascorbic acid diminished as compared to September 1955.

Physical Findings

The range of heights and weights of the subjects are presented in Table III.

The results of the physical inspection of various systems in regard to the presence of signs of possible nutritional deficiencies are presented in Table IV.

It is obvious that the percentage of students who presented various signs frequently associated with nutritional deficiencies was low, with the exception of seborrhea and hypertrophy of the filiform papillae of the tongue in both sexes and circumcorneal congestion of the conjunctiva in the men only. Whereas the examination of the women showed the complete absence of eleven signs of nutritional deficiency, the men showed such absence only in five instances.

Gross evidence of dental caries was high in both sexes (Table V).

The incidence of dental caries was higher and more severe in the men. All subjects had their carious teeth filled with the exception of six men. Two men and two women had partial dentures. The percentage of dental deformities was relatively low (14.7 per cent in the men and 9.7 per cent in the women).

COMMENTS

The adequacy of the diet of the Brazilian students was measured in Table II by comparing their mean intake of various essential

TABLE III

Range of Heights and Weights in Brazilian Students

No.	Subjects	Height (cm.)	Weight (kg.)
102	Men	154–178	42.0–80.5
31	Women	148–169	34.0–67.5

TABLE IV

Percentage of Students Presenting Signs of Possible Nutritional Deficiency in September–October 1955

Sign	Men (102)	Women (31)
Skin		
Xerosis	0	0
Follicular hyperkeratosis	12.8	25.8
Seborrhea	27.5*	58.1*
Eyes		
Blepharitis	3.0	0
Thickening of conjunctiva	19.6	9.7
Spots of conjunctiva	17.6	6.5
Follicular conjunctivitis	2.0	3.3
Circumcorneal congestion	45.1*	12.9*
Mouth		
Angular stomatitis	1.0	3.3
Cheilosis	0	0
Redness of tongue	0	0
Edema of tongue	18.6	12.9
Fissures of tongue	5.9	9.7
Atrophy of filiform papillae	2.0	0
Hypertrophy of filiform papillae	44.9	38.7
Atrophy of fungiform papillae	14.7	9.7
Hypertrophy of fungiform papillae	10.8	19.4
Redness of gums	9.8	0
Edema of gums	4.9	0
Bleeding of gums	1.0	0
Recession of gums	13.7	3.3
Neck		
Enlargement of thyroid	5.9	12.9
Skeleton		
Curvature of spine	0	3.3
Curved legs	8.8	0
Nervous system		
Diminished knee jerks	2.0	0
Exaggerated knee jerks	0	6.5
Muscular system		
Calf tenderness	2.0	0

* Differences statistically significant at 5 per cent level (χ^2 test used).

nutrients with N.R.C. recommended daily allowances for these same nutrients. N.R.C. allowances are intended for use in North Americans who are of larger average size than the Brazilians and live in a temperate climate.

TABLE V

Incidence of Dental Caries in 133 Students

Subjects	Absent (%)	Grade I (%)	Grade II (%)
Men	1.0	76.5	22.5
Women	22.6	67.7	9.7

TABLE VI

Comparison of Height and Weight of Brazilian and North American Students

Subjects	No.	Mean Height (cm.)	Standard Deviation (cm.)	Mean Weight (kg.)	Standard Deviation (kg.)
Brazilian Students (Belem)					
Men	102	167	5.4	60.7	9.7
Women	31	154	5.7	49.1	7.6
North American Students (New York)					
Men	70	172	6.8	74.5	8.6
Women	30	160	5.5	56.8	5.0

Physiologic requirements for these nutrients diminish in persons with decreased body size.

That the Brazilian students were considerably shorter and lighter than comparable North American students[4] can be seen from Table VI, presenting mean heights and weights for the two groups.

From this it follows that the metabolic needs of the Brazilians are lower than those of the North Americans. Therefore, North American standards such as the N.R.C. allowances are actually too high for Brazilian requirements— as they are for most of Latin America. However, because we do not have any standards for tropical Brazil as yet, these standard allowances have to serve with the aforementioned reservations. The intake of all nutrients presented in Table II must be considered adequate or better in both sexes.

The diet pattern of the Brazilian student may be considered typical for the upper class in Belem. It is similar in various aspects to the diet pattern of a group of Chinese students in New York studied by me in 1952–1953.[4]

TABLE VII
Comparison of Brazilian and Chinese Diet Patterns

Nutrient	Percentage of Total Calories	
	Brazilians (133)	Chinese (57)
Protein	9	16
Fat	22	38
Carbohydrate	69	46

This group of Chinese had adopted a diet pattern which was primarily American. The Brazilians, in comparison, ate more rice and beans and drank more coffee.

A comparison of the relative proportions of protein, fat and carbohydrate in the diets is made between the Brazilians and the Chinese[7] of both sexes in Table VII. In this comparison the fact that the Brazilians were eating considerably less protein and fat and correspondingly more carbohydrates becomes of great importance. This may be considered an advantage in the tropical heat because of the known specific dynamic increase of the basal metabolism caused by protein and the higher caloric value of fats. A mean daily protein intake of 54 gm. is to be considered adequate in the tropics, with 50 per cent derived from animal sources according to Brazilian authors.[5] The fact that the Brazilian intake of fats was only 22 per cent as compared to 38 per cent for the Chinese consuming an American-type diet (Table VII) becomes of particular interest in relation to the problem of atherosclerosis. There are no data available on the incidence of atherosclerosis in Brazil. However, some indication that it probably presents a serious problem can be gained from the data[1] on the high incidence of cardiovascular diseases among the parents of these students, which was about as high as in a comparable group of North American students.[4]

In view of the apparent adequacy of the diet in regard to various essential nutrients, a low incidence of physical signs associated with nutritional deficiencies could be expected in the Brazilian students. This was true in the majority of cases; however, the fact that such

signs as follicular hyperkeratosis, thickening and spots of the bulbar conjunctiva were found in a fair percentage of both men and women indicates that the intake of vitamin A was inadequate. I found a much higher incidence of the same eye signs in a low economic group on a much lower vitamin A intake[6] and an equally high incidence, however, of these eye signs and of follicular hyperkeratosis and xerosis of the skin in the Chinese and North American students in New York,[7] whose vitamin A intake was between 50 and 150 per cent higher than that of the Brazilian students. Such findings could indicate that these signs either are not pathognomic for vitamin A deficiency or that requirements for vitamin A are much higher than the N.R.C. allowances in certain groups. The high percentage of men with circumcorneal congestion of the eyes and hypertrophy of the filiform papillae of the tongue (the latter was also high in the women) cannot be explained on the basis of a low intake of riboflavin and niacin because the intake of these, even if one considers cooking losses, was more than adequate (Table II). The fact that circumcorneal congestion was significantly more frequent in men (as I have found in other groups) suggests that such factors as exposure to dust, wind and sun may be responsible.

Whereas conditions of the gums were generally satisfactory, the teeth showed evidence of a high rate of caries attack. Comparable groups of Chinese and North American students were found to have lower rates of caries than the Brazilians,[7] the Chinese showing the lowest rates. The mean daily sugar consumption per Brazilian student was 73 gm. in the men and 46 gm. in the women; this is lower than the national average given as 88 gm. per day.[8] It is also lower than the average for four North American cities in 1949, which was 96 gm. per person per day.[9] The percentage of sugar in the total calories was about 10 per cent among the Brazilians and the North Americans but less among the Chinese. It is clear that sugar consumption is high in Brazil as well as in the United States if compared with such countries as Haiti with 27 gm. per day per person[10] or Turkey with

25 gm. per day per person.[10] If it were still higher in Brazil, one would be justified in ascribing the higher caries rates to this higher intake of sugar; however, since this is not the case, other factors must be responsible for the higher and more severe incidence of caries among the Brazilians. That the total carbohydrate content of the diet is not a factor has been shown by such studies as that of Schour and Massler[11] in Italy where the total carbohydrate intake was high, but the sugar consumption low (during the years 1930–1934 the per capita daily consumption of sugar in Italy was 23 gm. as compared with 126 gm. in the United States).

The incidence of simple goiter was low in the Brazilian students with a higher incidence among women.

SUMMARY AND CONCLUSIONS

A total of 133 Brazilian medical students in the Amazon city of Belem have been examined in regard to their nutritional status between September 1955 and August 1956.

Dietary informations obtained from 104 students during one week showed a satisfactory to high mean intake of essential nutrients such as calories, proteins, calcium, iron, vitamins A and C, thiamine, riboflavin and niacin. The adequacy of these nutrients in the Brazilian diet was compared with N.R.C. recommended daily allowances. However, it must be remembered that the Brazilians have lower requirements as they are shorter in stature and lighter in weight than the North Americans.

The incidence of physical signs frequently associated with nutritional deficiencies was generally low, with the exception of such signs as hypertrophy of the filiform papillae of the tongue, circumcorneal congestion of the eyes (in men only) and follicular hyperkeratosis of the skin (in women only). Possible factors other than nutritional in the genesis of some of these signs have been discussed.

A high incidence of dental caries has been found in the Brazilian students, which was more severe among the men. Although sugar consumption is high, it does not explain the severe caries picture satisfactorily, since comparative groups of North American students with an equally high intake of sugar have a lower and less severe incidence of dental caries.

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Diet and Health of a Group of African Agricultural Workers in South Africa

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THIS REPORT deals with a study of the diet and health of a group of male African agricultural workers in South Africa made in February 1958. The workers, most of whom had been recruited in rural areas for full time employment for six months on a contract basis, were housed in barracks at their place of work.

Attention was directed to their clinical nutritional state and to their morale. The latter, which may be both a manifestation and a determinant of health, can be influenced by the satisfaction derived from food and by "somatic" nutritional factors.[1,2]

MATERIAL AND METHODS

The subjects were 118 workers aged twenty years or over, drawn from two barracks. Physical measurements of one of these men were inadvertently omitted. A few workers with occupational and possible dietary privileges ("policemen," "indunas" and cooks) were excluded.

Most of the men were Pondo (56 per cent) or Zulu (26 per cent). Although exact ages were not always available, it was estimated that 41 per cent were twenty to twenty-nine years old, 31 per cent were thirty to thirty-nine and 28 per cent were forty years or over. There were twenty-seven "new hands" in the sample, i.e., workers who had been in unbroken employment for less than two months, and ninety-one "old hands." Their previous employment had been urban (48 per cent), rural (27 per cent) or at this establishment (25 per cent).

Diet

A "household inventory" study was performed in the barracks, and a food habit questionnaire was given to the workers. The food consumed appeared to diverge little from the official ration scale, which was accordingly used as the basis for dietary appraisal. There was little waste of food or supplementation of the rations.

Clinical Nutritional Assessment

Detailed observations were confined to (1) selected skin and mucosal findings; (2) weight and height; and (3) skinfold thickness. Each man's skin and mucous membranes were examined simultaneously by two clinicians, who reached agreement in respect to each finding.

Skin Signs: Observations were confined to four skin areas, namely (1) face; (2) front of trunk; (3) back of trunk; and (4) buttocks and thighs. The shoulders, arms, knees and lower legs were excluded in view of their exposure to local traumatic influences. The skin signs sought were those listed in the next paragraphs. The intensity of each sign was scored for each of the four skin areas: 0, absent or minimal; 1, mild; 2, moderate; and 3, marked. Thus, four figures were recorded for each skin sign. The total of these four figures was regarded as the score for the sign, and used as a measure of the grade of the abnormality.

Three abnormalities of the pilosebaceous follicles were recorded:‡ (1) *phrynoderma* or follicular hyperkeratosis[3]; (2) *dyssebacia and/or*

‡ As there was little correlation between the scores for these three lesions, they were not combined into a single follicular index. There was a slight correlation between 1 and 3 (r = 0.16; p < 0.05), but none between 1 and 2, and a negative correlation between 2 and 3 (r = −0.39; p < 0.001).

TABLE I

Official Ration Scale and its Caloric Distribution

Food Item	Mean Daily Amount (oz.)	Per Cent of Total Calories
Cereals		77.6
Refined white maize meal	34.3	
Samp*	2.3	
Bran	0.6	
Sugar	2.9	6.6
Fruit and vegetables		5.6
Dried beans	3.4	
Potatoes	0.6	
Onions	0.02	
Meat	4.6	5.3
Beverages		4.9
Kaffir beer†	1.2 pint	
Coffee	0.1	
Salt	1.1	...

* Coarsely milled white maize product of 60 to 65 per cent extraction.

† A thin sweetish-sour fermented cereal gruel, with an alcohol content of about 3 per cent.[8]

TABLE II

Nutrient Content of Official Rations,* in Comparison with Recommended Standards†

	Official Rations	Recommended Standards
Calories	4,876	4,500
Protein (gm.)	142.6	65
Animal protein (gm.)	24.2	22
Calcium (gm.)	0.4	0.7
Iron (mg.)	43.8	9
Vitamin A (I.U.)	74.6	5,000
Thiamine (mg.)	1.7	1.6
Riboflavin (mg.)	1.2	1.6
Nicotinic acid (mg.)	20.6	18
Ascorbic acid (mg.)	9.4	40

* The food values used were those of Fox and Golberg.[9] Values for "mahewu" and beer were obtained from Golberg and Thorp[8] and Platt.[10]

† South African National Nutrition Council.[11] Standards for men (average weight 160 pounds) engaged in heavy work.

folliculosis,[3] including follicular enlargement (but not frank acne) on the face or in the sternal or interscapular areas; and (3) *follicular enlargement* or "permanent gooseflesh"[3] in skin areas other than those just mentioned.

The non-follicular skin signs recorded were: (4) *dryness;* (5) *dullness;* (6) *increased reticulation,* i.e., an accentuation of the normal reticulation of the skin, often with superficial crinkling; (7) *flaking;* and (8) *hyperkeratosis,* other than phrynoderma. The total of their five separate scores was used as an index of xerosis of the skin."*

Mucosal Signs: Observations were confined to the lips, gums and tongue, in which the signs listed subsequently in Table III were sought. The degree of abnormality of each of these three areas was clinically assessed as "none or minimal," "mild," "moderate" or "marked."

* There was some correlation among these five signs. The significant correlations, all positive, were: 4 with 5 (r = 0.96; < 0.001), 6 (r = 0.68; p < 0.001), and 7 (r = 0.20; p < 0.01); 5 with 7 (r = 0.22; p < 0.01); 6 with 7 (r = 0.49; p < 0.001) and 8 (r = 0.27; p < 0.001); and 7 with 8 (r = 0.23; p < 0.01). The total "skin xerosis" score was thus an index of skin dryness and dullness, and the commonly associated textural abnormalities.

These ratings were based on the nature, degree and extent of the signs found. Each of these mucosal ratings represented more than one separate process.†

Weight and Height: Workers were weighed unclothed. Height was measured with the line of sight horizontal. The relative weight of each man was assessed by comparing his weight with the mean weight of rural Zulu men of the corresponding height,[4] and also with the mean weight of American men of the corresponding height and age.[5] It was not possible to do this in all cases, as Zulu standards were not available for men below 61 inches or above 69 inches in height, or American norms for men aged over fifty-four. The American standards used were adjusted to approximate nude values by subtracting 1 inch for shoes and 8 pounds for clothing.

† There was little correlation among the various lip signs, the only association being between cracked and sodden angles (r = 0.17; p <0.05). Among the tongue signs, the only significant correlations were between papillary atrophy and, respectively, papillary enlargement (r = 0.16; p <0.05) and fissuring (r = 0.15; p < 0.05). Among the gum signs, there was positive correlation between marginal gingivitis, softness, swelling, redness and the presence of pus. Firm hyperplasia, however, was negatively correlated with marginal gingivitis, swelling and softness.

TABLE III

Prevalence of Skin and Mucosal Abnormalities Among 118 Workers

Abnormality	Prevalence (%)
Skin	
Follicular signs	
Phrynoderma	63.6
Dyssebacia and folliculosis	89.0
Follicular enlargement	66.9
Xerosis of the skin	
Dryness	100.0
Dullness	99.2
Increased reticulation	99.2
Flaking	61.0
Hyperkeratosis	66.9
Lips	
Dryness	82.2
Sodden angles	58.5
Cracked angles	34.7
Redness	2.5
Dry angles	2.5
Ulceration	0.8
Fissuring	. . .
Gums	
Marginal gingivitis	80.5
Pus	66.1
Firm, hyperplastic	44.1
Swollen	32.2
Softness	23.7
Redness	16.1
Bleeding	1.7
Tongue	
Indentation	94.9
Papillary atrophy	85.6
Fissuring	88.1
Redness	79.7
Papillary enlargement	16.1
Magenta hue	2.5

Skinfold Thickness: Measurements were made at three sites on the right side: (1) upper arm, halfway down the back of the arm over the triceps; (2) subscapular, immediately below the inferior angle of the scapula; and (3) suprailiac, immediately above the iliac crest. The site was marked, a fold lifted, and a Harpenden skinfold caliper[6,7] applied. Two measurements were made at each site by each clinician and a mean was calculated. If there was considerable difference between measurements, further observations were made.

Indices of Nutritional State: Eleven indices of nutritional state were used: phrynoderma, dyssebacia and/or folliculosis, follicular enlargement, xerosis of the skin, abnormalities of the lip, gum and tongue, relative weight and three skinfold thicknesses (arm, subscapular and suprailiac). These indices were not combined into a single index expressing the worker's total nutritional state as they showed little correlation.*

Morale and Productivity

Two indirect measures of morale were used: (1) the worker's absence rate; and (2) the number of items about which he complained in an interview. The former was expressed as the percentage of days on which he had been absent during his current employment or during the past year, whichever was less. Each man was asked a series of neutral questions regarding his food and other aspects of his work conditions. The interviews were conducted in the men's own languages by African health educators experienced in interview technic, and the men replied without restraint. The items about which each worker complained were recorded and counted. Each man was given two scores, one based on his complaints about food the second on his other complaints.

The work output of one category of workers was measured during a week's time by the management to provide an index of their productivity.

Other Observations

A rapid general physical examination was carried out to detect the presence of gross disease. This was supplemented by the records of the local medical service. In addition, stool and urine specimens were obtained from thirty-two and forty-two workers, respectively, and examined by the Amoebiasis Research Unit, Durban. Stools were submitted to direct examination and to zinc sulfate flotation. Detailed situational observations were made with particular reference to food and living quarters.

* Among the old hands, the only positive associations found were between skinfold thickness and relative weight, between the three skinfold measurements, between phrynoderma and follicular enlargement, between xerosis and follicular enlargement, and between gum and tongue signs. Dyssebacia and/or folliculosis was negatively correlated with xerosis and with follicular enlargement.

TABLE IV

Weights and Heights of the Workers, and Comparison
with Urban Zulu Figures
(Mean Values with Standard Deviations)

Group	No.	Weight (lb.)	Height (in.)
Workers	117	128.5 ± 14.7	64.76 ± 2.73
Urban Zulu men, aged 20 or over[13]	106	147.2 ± 27.9	65.4 ± 2.4

RESULTS

Diet

The workers' diet, although calorically ade-
quate, had serious qualitative shortcomings
(Tables I and II). Intakes of milk, meat,
fruit, vegetables and fats were absent or inade-
quate, and intakes of cereals were excessive,
maize in particular supplying 76 per cent of the
intake. Deficiencies existed in vitamin A,
ascorbic acid, calcium, riboflavin, and, if cook-
ing losses were taken into account, thiamine
and nicotinic acid.

A limited variety of dishes was served.
There were two formal meals a day, which al-
most always comprised a thick maize-meal
dish, sometimes served with beans or meat.
Apart from these meals, the main food avail-
able was "mahewu," a soured maize porridge
used as a beverage. Contamination of food
was frequent. Food was often exposed in un-
covered containers, and many flies settled on
exposed food. Eating and serving utensils were
few, and most workers ate with their hands.

Nutritional Findings

The prevalence of the skin and mucosal signs
is shown in Table III. No worker was com-
pletely free of the abnormalities sought.
Phrynoderma was found in 62 per cent of the
men examined, dyssebacia and/or folliculosis
in 88 per cent, follicular enlargement in 70
per cent and xerosis of the skin in all. Mild or
severe abnormalities of the lips were found in
71 per cent, of the gums in 70 per cent and of the
tongue in 99 per cent. Marked mucosal lesions
were infrequent. Signs of malnutrition other
than those listed were often observed, such as a
"buccal frieze,"[12] conjunctival heaping, "mo-

TABLE V

Skinfold Thicknesses (mm.) of the Workers (Median
and Mean Values and Standard Deviations), and Com-
parative Figures for Other Groups

Site	Group	Median	Mean
Arm	Workers	4.86	5.06 ± 1.76
	Durban Zulu men[13] aged		
	20–29	8.56	...
	30–39	8.38	...
	30–39	9.00	...
	50 or over	11.55	...
	Canadian men[14] aged		
	20–24	5.3	6.3
	30–34	7.3	8.2
	45–54	6.6	7.5
	English factory workers[15]	...	5.83
	Minneapolis fire-men aged 25–63[16]	12.5	...
Subscapular	Workers	7.10	7.22 ± 1.93
	English factory workers[15]	...	7.95
	Minneapolis fire-men aged 25–63[16]	16.5	...
Suprailiac	Workers	5.50	6.62 ± 2.29

NOTE: pressure at contact surface: 10 gm./mm.[2]

saic skin" on the legs and fissuring of the feet.
None had classic pellagrous dermatosis.

The mean weight of the men was much lower
than that of a population sample of urban Zulu
men, but their mean height did not differ sig-
nificantly (Table IV). Fifty-six per cent of the
men weighed less than the mean weight of rural
Zulu men of the corresponding height, and 75
per cent weighed less than the American stand-
ard weight for their height and age.

The observations of skinfold thickness are
summarized in Table V. The average arm
measurements were lower than those of urban
Zulu men in Durban, or of various overseas
groups. The proportion with arm skinfold
values below 9 mm. was 100 per cent, a figure
considerably higher (p< 0.001 in each instance)
than the corresponding figures for urban Zulu
men[13] (73 per cent), Cape Town Africans[16]
(60 per cent), Cape Town colored men[16] (56

TABLE VI

Skin and Mucosal Indices of New Hands and Old Hands

Nutritional Index*	New Hands (27)	Old Hands (91)
Phrynoderma	0.48	0.89
Dyssebacia/folliculosis	1.85	1.81
Follicular enlargement	1.07	1.11
Xerosis	10.33	12.01
Abnormalities of the lip	55.6	80.2
Abnormalities of the gum†	71.4	95.9
Abnormalities of the tongue	48.1	54.9

* In each case, the higher the figure, the more marked the abnormality. The skin indices represent mean scores for the respective lesions; the lip and gum indices represent the percentage of men graded as "mild" or worse, and the tongue index represents the percentage graded as "moderate" or "marked."

† These figures apply to the twenty-one new hands and forty-nine old hands aged thirty or more.

per cent) and various white groups cited by Brožek.[16]

Relation to Age, Ethnic Group, Previous Occupational History, and Disease Conditions

Among the old hands, who had been living under fairly uniform conditions for at least two months, there was clear evidence of nutritional variation with age. Men aged under thirty tended to have fewer mucocutaneous lesions, particularly of the gums and tongue, and men aged forty or over tended to have lower relative weights than younger workers, by comparison with either of the weight norms used. These age differences were found among both Pondo and non-Pondo workers.

There was no apparent relation between the nutritional findings and ethnic group, previous occupation or the length of the last break in employment.

There was apparently little major illness among the men. Apart from clinical cardiomegaly in one worker, no evidence of major illness was found. Bilharzia ova were found in only one specimen of urine. During the preceding year, fifty-six of the 118 men had attended the local hospital. The only serious illnesses diagnosed had been tuberculosis (in one man), pneumonia (in two), diarrheal disorders (in seven) and "fits" (in one).

Evidence of metazoal infestation was found in 50 per cent of the stools examined; the most common parasites were whipworm (in 38 per cent), roundworm (in 34 per cent) and hookworm (in 16 per cent). No relation could be demonstrated between infestation and the nutritional indices; however, the groups compared were small.

Relation to Period of Employment

The findings in the old hands differed considerably from those in the new hands. Among the old hands, phrynoderma and xerosis of the skin and abnormalities of the lip, gum and tongue were more common (Table VI). Four of these differences were statistically significant, those in phrynoderma ($p < 0.01$), xerosis of the skin ($p < 0.02$), lip signs ($p < 0.05$), and gum signs (among men aged thirty or more, $p < 0.02$). The difference in lip signs was due mainly to a higher prevalence of cracked or sodden angles, a slightly greater degree of dryness, and the difference in gum signs to redness, softness, pus and marginal gingivitis. There were no noteworthy differences between the workers employed for two to four months and those employed for longer periods.

The relative weights of workers in their third to fifth month of employment tended to be higher than those of men employed for shorter or longer periods. Of fifty-four workers in their third to fifth month, 41 per cent were below the rural Zulu norm; of twenty-four new hands, 67 per cent were below this norm ($p < 0.05$); of thirty-three men employed for over five months, 77 per cent ($p < 0.001$). This finding was to some extent paralleled by the skinfold thickness findings. Among workers in their third to fourth month of employment there were fewer men with low skinfold measurements than among workers employed for shorter or longer periods. In only one respect, however, was there a statistically significant difference, viz. between the proportions of new hands and men in their third to fourth month who had low suprailiac measurements ($p < 0.05$).

When age, ethnic group and previous occupational history were, in turn, held constant, these nutritional differences between old and new hands remained apparent. The size of

the groups compared did not warrant detailed analysis.

It was considered possible that these nutritional differences might be due to variations in the type of work performed by the old and new hands, respectively. Although all men were involved in strenuous work, their tasks were clearly defined, and could be categorized as "very heavy" or "relatively light." Separate examination of the findings in workers whose jobs were very heavy and those whose work was relatively light revealed similar differences between the new hands and old hands in each occupational group.

Morale

According to the management, there was much dissatisfaction among the men. This was manifested, *inter alia*, in a high turnover of labor and a high incidence of malingering. The workers' discontent was confirmed by our findings. Only 4 per cent had no complaints and 79 per cent had four or more complaints. Many complaints were forcibly expressed. Ninety-two per cent of the workers complained about their food and 96 per cent about other aspects of their work situation. Absence rates were high, 60 per cent of the men having rates of 5 per cent or more. The mean absence rate was 8.2 per cent, a figure which may be compared with the crude absenteeism rate of 3.58 per cent recorded for African workers in a Durban factory.[17] Records of the local hospital revealed that 35 per cent of the absences of the men studied had been associated with attendance for medical care. This would indicate a rate of absence due to sickness of 2.9 per cent. As many of the men stated, however, that they were reluctant to use their medical service, the rate of absence due to sickness was probably higher. The comparable figure for factory workers in Durban was 1.42 per cent.[17] This rate of absence due to sickness, taken in conjunction with the reported high incidence of malingering, was considered to be in part an expression of low morale.

The men engaged in particularly strenuous work had, by comparison with those performing lighter work, significantly more complaints about non-food items (p < 0.01) and higher rates of absence (p < 0.05).

Men with many food complaints tended to have many other complaints as well (r = 0.30; p < 0.01).

It was apparent that there was an objective basis for much of the men's dissatisfaction. Most workers complained, for example, about the range and variety of the foods offered, with apparent justification. Similarly, our observations indicated an obvious objective basis for many of their other complaints, such as those related to the preparation and serving of food, the living quarters and bedding ("We have to sleep on the sacks we have been wearing all day; sometimes they are wet.") and other aspects of the work situation.

Among the old hands, there was limited evidence of an association between their morale and the nutritional findings. In respect to every nutritional index used except dyssebacia and/or folliculosis, men with two or more complaints about their food had a worse nutritional state than those with one or no complaints. Some of the differences were, however, slight, and none was statistically significant. There was no consistent association between absence rates and the nutritional findings, although a single significant relation was found, between low absence rates and low skin xerosis scores (p < 0.01).

Productivity

The management was extremely concerned about the men's work output, which was unsatisfactory in spite of an incentive bonus system, and was interested in the possible influence of dietary factors on their productivity. However, no association could be demonstrated between productivity and the nutritional findings, absence rates or complaints.

COMMENTS

Skin and Mucosal Lesions: Clearly, the men were malnourished. Although many of the prevalent lesions were those which may be related to causes other than general malnutrition, the concurrence of a wide variety of lesions

in most of the men made non-nutritional explanations unlikely.

A high prevalence of malnutrition is not a surprising finding among South African workers. Lapping[17] found evidence of malnutrition in 99.3 per cent of one group of Durban African industrial workers, and Faure[18] in the majority of another such group. However, a comparison of our findings with Faure's strongly suggests that the degree of malnutrition was greater in the agricultural workers.

The malnutrition was probably primarily dietary in origin. Whatever the extra stresses to which the men were exposed, such as infestation and severe exertion, their diet appeared to be qualitatively inadequate to meet their needs. There is little value in any attempt to attribute the findings to deficiencies of individual nutrients. The lesions were compatible with the known effects of a predominantly maize diet similar to that of these workers, and were of a kind frequently observed among maize-eating populations.[19, 20]

The tendency for mucocutaneous lesions to be more marked in the older workers was not unexpected, in view of the cumulative and chronic nature of many nutritional changes. It indicated that the men's malnutrition was partly due to their experience before enlistment, and was not related solely to their current diet. The tendency for a wide variety of lesions to be more marked among the old hands indicated that these men were in a worse nutritional state than the new hands, and suggested that the workers' current diet had contributed significantly to the malnutrition. Had this been a longitudinal study, there would have been little difficulty in accepting this conclusion. In this cross sectional study, however, it was necessary to consider alternative explanations. The findings did not appear to be related to differences between the old and new hands in respect to age, ethnic composition or previous occupational history. It was possible, however, that they reflected variations existing in the men's nutritional state at the time of their enlistment, rather than the effect of the occupational situation to which they were subsequently exposed. Possibly as a result of seasonal factors, the condition on enlistment of the old hands had been worse than that of the new hands, and this difference had persisted until the time of examination.

Attention was accordingly focused on the seasonal nutritional variations occurring in the men's home areas. Ninety-three per cent of the men had lived in a "native reserve," mainly in Pondoland or Natal, during their last break in employment. Seventy-nine per cent had had a break of over three months, and 45 per cent of over six months. In Pondoland, where most had been recruited, and at the neighboring town of Tsolo, the incidence of pellagra reaches its peak in December to February,[21, 22] It was thus unlikely that at the time of their enlistment the old hands, 87 per cent of whom had enlisted between July and December 1957, had been more malnourished than the new hands, who had enlisted between December 1957 and February 1958. However, the seasonal incidence of malnutrition varies with place. In Pholela, Natal, for example, the prevalence of mucocutaneous lesions among school children is highest during July through December, and lowest in January through March.[20] Old hands recruited from such areas might in fact have been more malnourished, at enlistment, than new hands. The implication here was that the improvement which would have occurred in their nutritional state during January and February had they remained at home did not occur at work. Even in respect to such workers, therefore, it could be concluded that nutritional conditions at their place of work were worse than those in their home reserve areas. The inadequacy of these conditions in most South African reserves is well recognized.[23, 24]

Development of malnutrition appeared to be as marked among men performing relatively light work as among those more strenuously occupied. Physical exertion alone, although possibly an important factor, was thus probably not the major determinant of this development. As has been indicated, the lesions found were compatible with the diet consumed. The high caloric value of the diet might have further contributed to the development of these lesions by increasing the metabolic demand for essential nutrients.

The paucity of correlation among the muco-cutaneous indices (or between them and weight or leanness) confirmed the thought that persons exposed to fairly uniform conditions of diet and work may manifest malnutrition differently. This finding also underlined the difficulty of comparing the nutritional states of the individual members of such a group.

On the basis of the findings described, a series of changes in the rations were suggested along the lines recommended by the FAO[19] for the improvement of maize diets. Since the time of the study, dietary improvements have been effected by the management, and further changes are planned.

Weight and Skinfold Thickness: The men were light in weight by comparison with urban Zulu or American men. There was a fairly close correspondence with a rural Zulu standard, which, however, was based on a malnourished group. The skinfold measurements were low by comparison with urban Africans and various other groups.

The evidence suggested that after two months of employment, workers tended to gain fat and weight, and subsequently to lose fat and weight. As with mucocutaneous lesions, the possibility was considered that the apparent gain might reflect variations in the state of the men at the time of their enlistment. A seasonal variation in weight has been demonstrated in adults,[25,26] and under the conditions of privation often found in South African reserves, such variation is not unlikely. However, the heaviness of men in their third to fifth month of employment by comparison with new hands recently arrived from their homes suggested that the conditions at work were more likely to produce gain in weight than their home conditions.

This was readily explicable on the basis of the high caloric intake. Newly enlisted workers, probably offered more food than had been available in their homes, gained weight. Possibly, also, the development of muscular tissue in response to exertion contributed to gain in weight.

The subsequent loss of weight was difficult to explain. Newly enlisted men, having taken inordinate amounts of food for a period, might have then reduced their intake. It was the impression of a member of the management that this was in fact the case. Such a reduction might be related to the monotony or unattractiveness of the food. Although the effect of dietary monotony depends on the social culture,[27] it has been shown that if foods are constantly served, they tend to become less acceptable.[28] In experiments on volunteers who had been semi-starved for a period, Keys et al.[29] demonstrated a similar tendency for weight and body fat to increase after the period of semi-starvation, and then to decrease, although not to below the prestarvation values. Possibly, also, the qualitative inadequacy of the men's diet contributed to the loss of weight.

No reduction was advised in the caloric value of the diet in view of the possibility that this might reduce productivity.[30] Changes in the preparation and serving of the food were suggested which, together with the modifications recommended in the ration scale, were likely to enhance the palatibility of the diet.

Morale and Productivity: Although there was much dissatisfaction with the diet, it could not be inferred that this was necessarily the sole or main cause of the men's low morale. The evidence of lower morale among the men engaged in more strenuous work indicated the role of factors other than food. There was some tendency for men with many food complaints to have many other complaints as well. These findings conformed with Roethlisberger and Dickson's finding[31] that "the source of most employee complaints cannot be confined to one single cause, and the dissatisfaction of the workers, in most cases, is the general effect of a complex situation." There was much dissatisfaction, both with the food and with other aspects of the occupational situation, but it was not possible to assess the relative effects of each. It was apparent that there was an objective basis for much of this dissatisfaction.

An association between morale and nutritional state was not fully established and there was no evidence of an association between morale and productivity. Use of better indices of morale might have provided better evidence of such relations.

The absence of correlation between the nutritional findings and productivity was not sur-

prising. Actual output of work on the job is greatly influence by morale, which is in turn influenced by many non-nutritional factors.[2] Moreover, the nutritional variations in such a malnourished group were possibly not large enough to produce measurable differences in output. We were not able to confirm or deny that the workers' low output was a manifestation of malnutrition.

The effect of dietary modifications on morale and productivity could thus not be predicted. In the light of the total situation of the workers, and bearing in mind their occupational situation, their separation from their families and their other disabilities, it was possible that diet constituted a relatively minor source of their discontent. It was, however, clear that the diet was one source of dissatisfaction, and that the changes recommended might not only improve the men's nutritional state, but also lessen their discontent.

It was pointed out that such changes, if introduced in such a way as to promote their acceptance, might provide the workers with evidence of the management's goodwill, and could profitably serve as a starting point for the many other steps which a progressive management would be obliged to devise to improve morale and productivity.

SUMMARY

The diet of a group of male African agricultural workers in South Africa contains an excessive amount of maize and inadequate amounts of milk, animal tissues, fruit and vegetables. It is deficient in a number of essential nutrients. A limited variety of dishes is served, and food hygiene is poor.

There is a high prevalence of skin and mucosal lesions, of a type consistent with a predominantly maize diet. The men tend to be light and lean by comparison with urban Africans and various other groups.

There is evidence of a deterioration in the men's nutritional state after two months' employment. There is also evidence of weight gain after enlistment, with subsequent weight loss.

The morale of the men is low; this is apparently partly related to their unsatisfactory diet. Limited evidence is found of an association between morale and nutritional state, and none of an association between productivity and nutritional state or morale. It is considered, however, that dietary changes could usefully initiate a more comprehensive program designed to improve morale and productivity.

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Balance Studies on Peanut Biscuit in the Treatment of Kwashiorkor

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GENERAL agreement exists on the importance of a deficiency of protein in the diets of young children, and increasing conviction is that the deficiency is often accompanied by a shortage of calories. In countries that have a well developed dairy industry, the standard method of avoiding deficiency is the use of cow's milk as the supplement to the rest of the diet. Elsewhere, there is need for other foods that can be used in the same way as cow's milk and have similar effects. The ingredients should be indigenous and inexpensive and the final product should be readily acceptable. For a tropical country, it should keep well in conditions of high temperature and humidity and, to neutralize uncertainties in the quality and quantity of the rest of the diet, it should provide plentiful calories as well as protein.

A dry powder has obvious advantages, and in Uganda we have been trying to perfect a powder that has for its chief ingredients the two inexpensive foods that are most easily available locally, peanuts and corn. We have added cottonseed oil, which is well tolerated even by children in advanced states of protein deficiency[1] and which seems to have a remarkable capacity for inhibiting spoilage by molds and other noxious agents, sugar because it is sweet and a small amount of wheat flour because its gluten is a convenient binder. The mixture of amino acids so provided cannot, by any adjustment of relative quantities, be made to resemble closely the mixture in cow's

milk, and we therefore added dried skim milk to our mixtures, realizing that it would be of value not only for its protein but also for its minerals. The ingredients were cooked into biscuit, which was hammer-milled into a fine powder. Details of preparation are given later.

The powder is in evolutionary descent from the soybean cereal mixtures that were tested in Germany after World War II and that were improved by small amounts of added milk.[2] The only other mixture with similar qualities that has been tried at all extensively is that made by the Institute of Nutrition of Central America and Panama (INCAP) in Guatemala City. It contains corn, sesame meal, cottonseed press cake, torula yeast and kikuyu leaf meal. It appears to be regarded favorably, but few details of its use have been published. In five children the retention of nitrogen from the mixture was 17 per cent of the intake, the same as from a milk diet.[3]

Several peanut mixtures with their ingredients in different proportions and cooked in various ways have been tried under strict conditions of control, in the feeding of children admitted for the treatment of kwashiorkor to the wards of the Infantile Malnutrition Research Unit. The effects of the mixtures in the first weeks of treatment have been compared with those produced by a diet in which all the protein was derived from cow's milk, the chief criteria used being clinical improvement, especially the loss of edema, gain in weight and alterations in the serum chemistry. A complete account will be published elsewhere. The present paper gives the results of comparison of the nitrogen retentions from the milk diet and the biscuit mixtures when they were used during the convalescence of children who had been treated for at least fifteen days,

and whose serum chemical findings were apparently normal. The nitrogen balance technic was used, and the opportunity was taken for some studies of fat absorption, nitrogen partition in the urine and alterations in blood urea. The thorough trial of a new diet occupies the greater part of our clinical and biochemical resources for about a year, and one reason why the balance experiments were undertaken was that we hoped to show whether or not we could economize in the number of trials needed for the development of a satisfactory biscuit.

The general plan was that each child be given two balance periods so that his performance on different diets could be assessed. It was intended that the nitrogen intake per kilogram of body weight should be steadily maintained throughout each period, and should be the same in both periods. However, this ideal could not be attained exactly.

It soon became obvious that biscuits made by cooking all ingredients together gave results that were inferior to those obtained from the milk diet, although biscuits and milk provided the same amounts of total protein. One possible cause was that some of the amino acids of the biscuit protein were being damaged, or made unavailable, by the heat of cooking.[4] It seemed likely that the protein of the dried skim milk was being deprived of some of its value, and to test this theory, the ingredients of the biscuit were cooked without the milk, the biscuit was powdered and the milk then added. The resulting product gave excellent results, but its success made necessary a further series of experiments to decide the most economic level of addition for the milk.

MATERIAL AND METHODS

The children were boys from eighteen to thirty-six months old who weighed from 5.5 to 9.5 kg. The diagnosis of kwashiorkor was unequivocal in every case at the time of admission, but there was considerable variation in severity: six cases were classed as advanced, eight as moderate and eight as early. The balance beds which have been in use in the Unit for several years, allow for the separate collection of urine and feces, the one through a glass tube that encloses the penis and scrotum and the other in an enamelled pot. A harness around the trunk and legs limits movement but does not entirely prevent it, and there are devices that raise the upper part of the body and the knees so that during the day the child can sit in comfort and at night can lie flat. Few of the children were made ill at ease by the restraint; most were obviously happy on the beds because they received special and continuous attention.

To standardize the effects of the enforced rest,[5] every child was kept on his balance bed for two or three days before the collection of urine and feces began. The beginning of each experimental period was marked by administration of carmine before a meal at 5 A.M. The first carmine-stained stool was discarded and all subsequent stools were saved. A second marker was given ninety-six hours after the first, also with the 5 A.M. meal and the passage of the first stool marked with carmine ended the period. The collections of urine ran from the time of appearance of the first marker to the time of appearance of the second. The accuracy of all balance methods depends to some extent on the regular voiding of feces, which could not, of course, be assured in our children. Extending the length of the periods reduces the importance of inaccuracies, but two four-day periods necessitated a total of fourteen days continuously on the balance bed, and we believed that to be long enough for the children and the staff.

Throughout the balance periods, the children were fed at 5 and 9 A.M., and at 1 and 5 P.M. All meals were given by the Unit's nurses, who also washed the children, made all the adjustments needed to the bed and harness and were responsible for the collections. The wards of the Unit have large glass windows, and the children were under continuous observation.

At the end of the first balance period, the diet was changed, and two or three days on the new diet was always allowed before the second balance period was started. One child had three periods, with the usual interim between the second and third.

Each child was weighed, and bled from the

internal jugular vein before and after each period. Except for these operations, which took only a few minutes, no child left his bed between the beginning of the first period and the end of the last. There were usually small changes of weight during the periods (Table II). For the results that were related to body weight, the lowest weight recorded at the beginning or the end of a period was used; the lowest was usually at the beginning.

All estimations were made on a daily basis. The stools and urines were collected in glass jars at twenty-four–hour intervals, and were acidified with hydrochloric acid; the urines were kept under toluene. All stools passed in each twenty-four–hour period were combined and prepared for analysis by adding water to a known volume and mixing in a high-speed electric blender. Each twenty-four–hour collection of urine was kept separately. The twenty-four–hour collections became of special value in those few instances in which the balance periods had to be shortened to three days. The shortened periods are indicated, but the results they gave agreed well with those for the longer periods.

Any vomitus was saved and analyzed, and the necessary deductions were made when the value of the food intake was calculated.

The chemical methods used were as follows:

For Food: For nitrogen estimations, samples were digested with concentrated sulfuric acid, using copper selenite as a catalyst. The Markham apparatus was used for the micro-Kjeldahl distillation into boric acid, and Tashiro's indicator was used for the titration. Fat was estimated by the method of von Liebermann, as described by McCance and Shipp.[6] The method was chosen because it is probably the best available for plant materials.[7]

For Stools: The micro-Kjeldahl procedure, as for food, was used for nitrogen, and the method of van de Kamer[8] for fat.

For Urine: Nitrogen was estimated by the micro-Kjeldahl method and urea by the method of Archibald.[9] The method of Van Slyke and Cullen, as modified by Hawk, Oser and Summerson,[10] was used for ammonia. One change was made in that the ammonia was removed by aeration after the addition of 10 N sodium hydroxide instead of saturated potassium carbonate so that after adjustment to pH 9.2 the ammonia-free extract could be used for a rough assay of amino nitrogen by the method of Frame, Russell and Wilhelmi.[11]

In our previous observations variations in serum urea of children who were being treated for kwashiorkor were so large that they could not justifiably have been ignored if nitrogen retentions had been calculated. In our balance experiments we therefore estimated the urea (by Archibald's method) in the serum samples obtained before and after each period. The differences were usually small and have not been taken into account in the calculation of the retentions.

Conversion Factors: In the calculation of caloric values the factors 4.1, 9.3 and 3.75 were used for protein, fat and carbohydrate, respectively. The factor 6.25 was used throughout for the conversion of nitrogen to protein.

DIETS

For the first twenty-five balances, the order in which the diets were given was varied to cancel any effect that might be due to changes in the clinical condition of the children. The length of treatment before the first balance, and the normal condition of the serum chemistry regarded as an essential prerequisite for selection, probably made this precaution unnecessary. No difference in the results could be detected that might have had its origin in the choice of the order.

The standard of reference was the milk diet, in which all protein was derived from cow's milk, and which long experience in the Unit's wards has proved to be entirely satisfactory in the acute stage of kwashiorkor and in convalescence. It varied slightly in the amounts of its ingredients but was most often made in the following manner: 20 gm. calcium caseinate (Casilan, Glaxo), 25 gm. dried skim milk (a spray-dried powder supplied by the Protein Committee of the National Academy of Sciences), 20 gm. sugar (sucrose from sugar cane) and 40 gm. cottonseed oil (a refined product of local manufacture) were mixed with

TABLE I

Composition and Value of Biscuits Used in Balance Experiments

	Biscuit			
	8C and 8U	15C and 15U	26U	8HC
Peanuts (gm./100 gm.)	48.0	41.0	35.7	40.0
Corn flour (gm./100 gm.)	20.0	20.0	17.4	20.0
Wheat flour (gm./100 gm.)	8.0	6.0	5.2	..
Cottonseed oil (gm./100 gm.)	4.0	6.0	5.2	4.0
Cane sugar (gm./100 gm.)	12.0	12.0	10.4	12.0
Peanut flour (gm./100 gm.)	..	..	..	16.0
Dried skim milk (gm./100 gm.)	8.0	15.0	26.1	8.0
Total protein	20.5	20.4	22.2	24.8
Total protein from milk protein (%)	13.4	25.1	40.1	11.0
Calories/100 gm.	476	492	473	487
% total calories from				
Total protein	17.5	17.0	19.2	20.7
Milk protein	2.4	4.2	7.6	2.3
Fat	45.6	47.6	43.1	45.7
Calories/gm. protein	23.3	24.1	21.3	19.6

water in an electric blender, and made up to 1,000 gm. with additional water.

Each 100 gm. of the mixture provided 2.6 gm. protein, 3.4 gm. carbohydrate and 4.1 gm. fat, with a caloric value of 61, and a ratio for calories: gm. protein of 23. Of the total calories, 17.5 per cent were derived from protein and 62 per cent from fat. The diet was a fluid resembling fresh milk in consistency. It was made daily and kept refrigerated in a jug. The oil tended to separate on standing, and before a feed was given the contents of the jug were thoroughly stirred.

The ingredients of the various biscuit mixtures are shown in Table I. Locally grown peanuts (groundnuts) of the Valencia variety were used. They were decorticated, but not roasted and their red skins were not removed; they were prepared merely by grinding in a simple machine that had a rotating cylinder carrying small raised cutters like those of a cheese grater. A device held the seeds against the cylinder until they were reduced to fairly small particles. The machine, originally devised for making poultry food from kitchen wastes, was a Gemuba bone and turnip mill (supplied by Union Food, Machinery and Equipment, Ltd.). It is normally worked by hand, but we fitted a small electric motor. The corn and wheat flours were of about 70 per cent extraction, and the sugar and cottonseed oil were the same as were used in the milk diets. The ingredients were stirred in a large Hobart mixer, with enough water to ensure thorough mixing. One mixture (8C) was cooked in a nearby commercial bakery. The dough was rolled out there and cut into rounds that were baked in a bread oven at about 200°c. All other mixtures were cooked in the Unit's special kitchen. The stiff dough was rolled into slabs about 1 cm. thick on trays 30 cm. by 50 cm. with a midline division. These slabs were cooked in a pastry oven at 200°c., for fifteen minutes on one side and five minutes on the other. The baking time and the temperature were those found by experiment to be the least that could be relied upon to give an even, well cooked biscuit. Finally, the biscuit was broken into small pieces which were then passed into a hammer mill fitted with a fine mesh screen.

In the first batches of biscuit the dried skim milk was cooked with the other ingredients. Later batches were made similarly, but as already mentioned, the milk was omitted from the cooked mixture. It was stirred into the hammer-milled powder in the Hobart mixer as the final process.

As shown by Table I, the milk was cooked into biscuits 8C, 15C and 8HC. It was not cooked, but added raw, for biscuits 8U, 15U and 26U. (In the code names that have been used, the numeral shows the percentage of milk, C indicates that the milk was cooked and U that it was uncooked.)

It is necessary to explain why so many different biscuit formulas were tested. The first (8C), which had 8 per cent dried skim milk, did not give satisfactory clinical or biochemical results, and was modified by the increase of the amount of the dried milk to 15 per cent (biscuit 15C). About the same time, a fat-extracted peanut flour became available and a

TABLE II
Results of Balance Experiments

Case No.	Diet*	Intake			Excretion				Nitrogen Retention (mg./kg./day)	Fat Absorption (%)	Weight Change (gm./day)
		Nitrogen (mg./kg./day)	Fat (mg./kg./day)	Calories (cal./kg./day)	Fecal Nitrogen (mg./kg./day)	Urine Nitrogen (mg./kg./day)	Urine Nitrogen: Fecal Nitrogen	Fat (mg./kg./day)			
7	Milk	582	4,330	94	58	372	6.4	510	152	88.3	+44
2	Milk	704	...	76	74	467	6.3	...	164	...	+17
6	Milk	734	6,400	127	93	331	3.6	670	310	89.5	+80
8	Milk	751	4,960	96	46	520	11.3	289	185	94.2	+57
1	Milk	783	4,030	98	130	551	4.2	742	103	81.5	+92
12	Milk	819	6,980	139	115	512	4.5	488	192	93.0	+76
13	Milk	863	7,810	145	116	642	5.5	796	105	89.8	+82
5	Milk	921	8,060	149	148	493	3.3	1,430	281	82.2	+33
9	Milk	940	6,250	131	94	603	6.4	746	243	88.1	+70
10	Milk	999	6,280	128	127	706	5.6	616	166	90.2	+92
11	Milk	999	6,950	137	95	627	6.6	611	277	91.2	+97
3	Milk	1,546	5,150	120	103	1,247	12.1	396	197	92.4	−40
7	8C	556	3,710	102	131	368	2.8	281	58	92.5	+16
6	8C	575	3,800	107	138	385	2.8	257	52	93.2	−10
1	8C	660	5,300	105	188	401	2.1	395	74	92.6	−10
5	8C	708	4,710	133	252	384	1.5	413	72	91.2	+23
2	8C	723	...	114	234	392	1.7	...	97	...	− 7
2	8C	829	6,780	131	150	510	3.4	260	169	96.2	+32
4	8C	840	...	148	151	434	2.9	...	254	...	+33
1	8C	859	...	145	190	401	2.1	...	268	...	− 6
12	15C	802	5,560	133	224	497	2.2	365	81	93.4	+33
11	15C	886	6,300	141	244	540	2.2	525	101	91.7	+35
8	8HC	805	4,840	83	183	565	3.1	294	57	94.0	−20
9	8HC	876	4,920	117	247	501	2.0	499	128	89.9	+23
10	8HC	990	6,140	137	180	660	3.7	322	151	94.8	+68
21	8U	626	4,760	96	97	467	4.8	164	62	96.5	+ 5
22	8U	712	4,680	123	127	476	3.8	331	109	92.9	+56
20	8U	740	5,380	123	144†	470†	3.3†	355	127	93.3	+30
19	15U	590	3,710	101	102	358	3.2	227	130	93.9	+28
21	15U	667	4,350	109	114	353	3.1	371	200	91.5	+52
15	15U	671	5,260	117	155	375	2.4	302	141	94.3	+57
14	15U	704	5,480	124	117	476	4.1	273	111	95.0	+30
22	15U	714	4,830	122	107	459	4.3	556	148	88.4	+50
16	15U	727	6,040	122	122	470	3.9	825	135	86.3	..
13	15U	757	6,400	117	179	453	2.5	692	125	89.2	+80
18	15U	778	6,130	135	163	410	2.5	349	206	94.3	+68
17	15U	790	6,200	137	91	606	6.7	267	94	95.7	+68
20	15U	830	5,370	138	122	525	4.3	452	184	91.6	+66
13	15U	906	7,370	150	224	528	2.4	854	155	88.4	+49
19	26U	640	3,950	99	120	452	3.8	311	69	92.1	+29
15	26U	744	5,230	138	182	430	2.4	506	133	90.4	+14
14	26U	745	5,210	118	122	504	4.1	392	119	92.5	+62
18	26U	782	5,460	125	233	424	1.8	417	124	92.4	+ 7
17	26U	790	5,580	124	104	604	5.8	365	81	93.6	+ 8
16	26U	853	6,080	134	144	599	4.2	533	110	91.2	...

* If biscuit diet was given, only number is shown.
† Figures approximate because one sample of stools was contaminated with urine.

mixture containing it (biscuit 8HC) was made. The first biscuit made with uncooked milk had 15C. The other biscuits had 8 per cent of the uncooked milk (biscuit 8U) or 26 per cent (biscuit 26U). They were used to indicate the optimal amount of the added milk.

It was intended that 100 gm. of all biscuits should provide about 20 gm. protein and 480 calories, with the ingredients in approximately the same proportions, and the ratio of calories to protein near that of the milk diet. It was further intended that all diets should be fed

in amounts that would provide 600 to 900 mg. nitrogen/kg. body weight/day, that is, approximately 3.75 to 5.50 gm. protein/kg. body weight/day.

The biscuit meals were made into a thin gruel with water, usually with one part of the meal to three parts water. All children having the milk or the biscuit diets were allowed up to 200 gm. daily of either mashed sweet banana or cooked banana (plantain). However, they did not regularly take this amount, and the protein of the bananas was never more

TABLE III

Results of Balance Experiments in which Nitrogen Intake Was Between 550 and 900 mg./kg. Body Weight/Day

Diet	No. of Balances	Average Nitrogen Intake (mg./kg./day)	Average Nitrogen Retention (mg./kg./day)	Range (mg./kg./day)	Average Retention (%)
Milk	6*	750	150	103–192	20.0
Biscuit 8C	5	666	85	52–169	12.8
Biscuit 15C	2	844	91	81–101	10.8
Biscuit 8HC	2	841	93	57–128	11.1
Biscuit 8U	3	693	99	62–127	14.3
Biscuit 15U	10	723	147	94–206	20.3
Biscuit 26U	6	759	106	69–133	14.0

* One child (Case 6 in Table II) who retained 310 mg. nitrogen from an intake of 734 mg. nitrogen, has been omitted. See text.

than about 7 per cent of the total protein intake.

RESULTS

The results are given in full in Table II. Of a total of forty-two balances, forty-one have been included in an analysis of nitrogen retention by average and by paired comparisons. One balance on a child given an extremely high intake of milk was excluded from the comparisons. Three balances on children who had been treated for less than fifteen days have been added to this table. The four balances have been dealt with in a separate section.

There were five balances that had to be curtailed to three days (Case 10 on biscuit 8HC, Cases 17, 21 and 22 on biscuit 15U and Case 15 on biscuit 26U). The rest ran the full four days.

Nitrogen Retention

Milk Diets: Average Retentions: The nitrogen retentions in the balances on the milk diet showed a slight tendency to increase with the intake. The intakes from the biscuit diets were nearly all under 900 mg./kg. body weight, and it seemed reasonable to take as the average standard for comparison the retentions from the milk intakes that were also under that figure. There were seven, and they were 103, 105, 152, 164, 185, 192 and 310 mg./kg. body weight. The average of the first six is 150 and of all seven, 173.

The averages obtained from all the results of the experiments in which the intakes were under 900 mg./kg. body weight are given in Table III.

Biscuit Diets: Average Retentions: For the three kinds of biscuit in which dried skim milk was cooked with the other ingredients (8C, 15C and 8HC) there was a total of nine balances in which nitrogen intake was less than 900 mg./kg. body weight, and eight showed retentions of 52 to 128 mg./kg. One child on biscuit 8C had a retention of 169 mg./kg., but the four other balances on this biscuit gave retentions of 52, 58, 72 and 74 mg./kg. The average of the four (64) probably gives a better indication of the value of the biscuit than the average of the five (85), but both averages are low.

Of the three kinds of biscuit to which the dried milk was added uncooked, biscuit 15U was given the most extended trial. The results were reasonably consistent and bore comparison with those for the milk diet; their average was 147 mg./kg. Neither biscuit 8U nor 26U gave such high retentions as biscuit 15U.

Comparisons of Different Diets Given to the the Same Child: Although the average results may arouse the sympathy of other investigators who have faced the difficulties and hazards of balance studies on children of this age group, they cannot be called convincing. Our previous experience on the variability of balances had prepared us for such a conclusion

TABLE IV

Comparison of Nitrogen Retentions Found in Children Given Two of the Test Diets

Case No.	Diet*	Nitrogen Retention (mg./kg./ day)	Diet*	Nitrogen Retention (mg./kg./ day)
1	Milk	103	8C	74
2	Milk	164	8C	169
5	Milk	281	8C	72
6	Milk	310	8C	52
7	Milk	152	8C	58
8	Milk	185	8HC	57
9	Milk	243	8HC	128
10	Milk	166	8HC	151
11	Milk	277	15C	101
12	Milk	192	15C	81
13	Milk	105	15U	140
14	15U	111	26U	119
15	15U	141	26U	133
16	15U	135	26U	110
17	15U	94	26U	81
18	15U	206	26U	124
19	15U	130	26U	69
20	15U	184	8U	127
21	15U	200	8U	62
22	15U	148	8U	109

* If biscuit diet was given, only number is shown.

and had led us to use each child as his own control by giving him periods on different diets. The results of nineteen pairs of estimations are shown in Table IV. For these pairs the restriction to intakes of below 900 mg./kg. body weight has not been applied. The results can be summarized as follows: (1) milk vs. biscuit 8C: the retention from milk was greater in four balances, and the same as from biscuit 8C in the fifth; (2) milk vs. biscuits 15C and 8HC: the retention from milk was greater in all five balances; (3) milk vs. biscuit 15U: the figure for retention from the biscuit (140 mg./kg.) is the mean of two balances, that gave results of 125 and 155 mg./kg. on one child; and (4) biscuit 15U vs. biscuits 8U and 26U: the retention from biscuit 15U was greater in seven children. Two children had approximately equal retentions on biscuits 15U and 26U.

The results show, more clearly than the average figures, the superiority of the milk diet over the biscuit diets containing the cooked milk, and the superiority of the biscuit 15U

over the other biscuits containing smaller and larger amounts of the uncooked milk. There were no comparisons, in the same child, of biscuits containing cooked and uncooked milk, but the success of biscuit 15U made such comparisons unnecessary.

The percentage of the nitrogen intake that was retained is shown in Table III. It was 20 for the milk diets and for biscuit 15U, but only 11 to 14 for the other biscuits.

Results of Balances Omitted from the Averages and the Paired Comparison: One child (Case 3 in Table II) had an intake of 1,546 mg./kg. from the milk diet in one balance period, and a retention of 197 mg./kg. A balance on biscuit 8C was carried out on another child (Case 4), but it started on the tenth day after admission before the child was fully convalescent, and it gave the high retention value of 254 mg./kg. A similar value of 268 mg./kg. was obtained from another child (Case 1), whose first balance on biscuit 8C started on the seventh day, but a balance on the same biscuit on a third child (Case 2), who started on the eighth day, gave a retention of only 97 mg./kg.

Nitrogen in the Urine and its Partition: The ratios of urinary nitrogen to fecal nitrogen, included in Table II, were consistently higher in the balance periods on the milk diets, but the partition of the urinary nitrogen did not vary greatly with the kind of diet. The full results of the estimations of urea, ammonia and amino nitrogen will be published later; they will be only summarized here. The percentage of the total urinary nitrogen that appeared as urea nitrogen varied from 85 to 95. Neither the milk diet nor the biscuit diets consistently produced a high percentage, and in nine children, the percentage was almost exactly the same in both balances. This occurred in Cases 5, 8, 10, 11, 12, 14, 15, 17 and 18 (Tables II and IV). The first five had a milk diet in one balance period and a biscuit diet in the other and the rest had two biscuit diets. There was a tendency towards a greater excretion of ammonia and amino nitrogen when the milk diet was being given, but the variations were large and were not related to the nitrogen intake or to other factors. The

different biscuit diets contained widely ranging quantities of milk protein, but they did not produce a similar tendency.

Fat Absorption

The average absorption of fat is also included in Table II. The average percentage absorp tion from the milk diets was about 89 per cent. Slightly higher percentages, 92 to 94, were absorbed from the biscuit diets, but the total intakes from the milk diets were slightly higher than those from the biscuit diets. The difference between the average absorption from the milk diets and that from all biscuit diets is statistically significant, p being greater than 0.01, although the difference between the intakes is not significant at this level.

There was no obvious correlation between fat absorption and nitrogen retention. One child (Case 9), who had slightly soft stools but whose clinical condition did not appear to change, showed a marked fall in fat absorption in the third and fourth days of his balance on a milk diet, but the nitrogen retention was affected only slightly.

The ratio of fat to protein in the stools was about 1.0 for the children on the milk diet, and 1.8 for those on biscuit 15U. It was 3.3 for the children on biscuit 15C, further proof, presumably, of the inferiority of that biscuit. The results were the more striking because the ratio of fat to protein in the milk diet was 1.5, but only 1.25 in the other diets. The different proportions of fat and protein in the stools could not be accounted for merely by the better absorption of the protein of the milk diet. The possible significance of differences in the ratio of fat to protein in stools will be explored further.

COMMENTS

The balance experiments confirmed the value of biscuit 15U. The biscuit has been given a long trial in the treatment of kwashiorkor of all grades of severity, and has been found to be almost equal to the milk diets in its ability to remove edema, to promote weight gains and to cause rapid and desirable alterations in serum chemistry. By the time the balance experiments were begun, the serum

chemical findings were always normal by the standards of healthy local African children of the same age, and the regular routine examinations of the serum showed no significant fluctuations when the diets were changed. The environment of the type of balance study described here is so artificial that, in the past, we have taken little notice of gains or losses in weight during the experimental periods, but eleven of the twelve children who had the milk diets gained (Table II), as did ten of the eleven who had biscuit 15U. On both diets, the average gain was about 55 gm./day. In general the gains of the children who had the other biscuits were less consistent and smaller.

The choice of the value of 20 per cent for the total protein in the biscuits represented a series of compromises between proportions and cost of ingredients, weighted by other considerations such as the desire to achieve a high calorie:protein ratio, the need for the product to have an acceptable taste and the capacity of a child's stomach. Within these limitations, our chief problem has been to make the best use of the protein and to improve the quality of the protein in the most economical way.

There is no doubt that much experimental work intended to define the biologic value of proteins has given fallacious results because insufficient calories were provided. It is, however, extremely difficult to determine optimum calorie:protein ratios. In breast milk the ratio is about 50, but in well varied diets taken by British and American children one to four years old, and satisfying the needs for good health and growth, the ratio is about 8.[13,14] The ratio in biscuit 15U was another compromise: it was 24. It may not be ideal, there may in fact be one ideal ratio for the diet for the treatment of acute kwashiorkor, and another for a convalescent diet, etc., but a ratio of 24 seemed at least likely to ensure that protein was not used primarily as a source of calories. It may be a suitable ratio for a food intended as a supplement to a low protein basic diet.

The most obvious way of improving the quality of the biscuit was by the addition of

dried skimmed milk. The milk was regarded as a valuable ingredient, but it was the most costly, even in terms of protein, and economy was essential. We have been fortunate in our choice of 15 parts in 100 for biscuit 15U. Biscuits 8U and 26U, which contained less and more of the milk, respectively, were less successful in promoting nitrogen retention. The comparisons of biscuits 15U and 26U were especially important. The failure of biscuit 26U to show a clear advantage might mean that the limit of usefulness of the milk in the conditions of our experiments had been reached. An excess of lactose can sometimes cause diarrhea[15] and we thought it possible that the increase in the intake of lactose brought about by the change from biscuit 15U to biscuit 26U, although small, might have altered conditions in the intestine in such a way that the nitrogen absorption was reduced. We could, however, calculate the absorption for the six children given biscuit 15U first, then biscuit 26U, and it was almost exactly the same in five of them; in only one was it higher on biscuit 15U. Therefore we must look for some other explanation of the failure of biscuit 26U.

The balances showed with a fair degree of certainty that a reduction of the protein value of the biscuits occurred when the dried skim milk was cooked with the other ingredients. When the milk was added uncooked, the nitrogen retentions were improved. It is known that one way in which heat damages protein is by rendering certain amino acids unavailable for biologic processes, and that lysine is often so affected. The mechanism, in lysine, appears to be the blockade of the free E-amino groups, and we have been able to show that the less efficient mixtures 8C and 15C contained 30 per cent fewer of the free groups than their more efficient counterparts 8U and 15U. The causative agent appeared to be the lactose of the dried skim milk.[4] The results agree with the findings in the balance experiments and suggest several interesting fields of study, for instance, an examination of the value of the protein of foods in which dried skim milk is cooked at a high temperature, and experiments in the addition of lysine to foods, either as the pure synthetic *l*-acid, or in an intact

TABLE V

Comparison of Amounts of Amino Acids in Two of the Diets Used in the Balance Experiments, and in Human Milk*

Acid	Milk Diet	Biscuit 15C	Human Milk
Arginine	3.7	8.6	2.9
Histidine	2.9	2.4	1.9
Isoleucine	6.0	4.9	5.0
Leucine	10.2	8.4	9.2
Lysine	8.0	4.5	5.9
Methionine	2.6	1.4	2.2
Cystine	0.6	1.3	1.5
Phenylalanine	5.6	5.0	3.8
Threonine	4.4	3.3	4.4
Tryptophan	1.3	1.2	1.8
Valine	6.9	5.2	5.0

NOTE: The values for the amino acids have been taken from the tables of Harvey, and the milk diet is that for which details are given in the text.

* Gm. amino acid/16 gm. nitrogen.

protein. High temperature cooking has many advantages, and its disadvantages might be tolerated if they could be easily and cheaply overcome.

Although our chief concern has been in the comparison of the various biscuits, it is interesting to make a further comparison of the total proteins of the most successful biscuit, 15U, with those of the milk diet. The most striking difference between the two diets was in the percentage of the total calories that came from milk protein: 17.5 in the milk diet (in which it was the only source of protein) and 4.2 in the biscuit diet (in which it provided one-fourth of the protein). There are large differences in the proportions of the essential amino acids in milk and vegetable proteins. The physiologic importance of the differences is an almost completely unsolved problem, but if we can regard the conditions of our experiments as physiologic, the results with the milk diets and biscuit 15U, should be of aid in a solution or a definition of minimum requirements for the amino acids.

The amounts of the amino acids in the milk diet usually employed and those in biscuit 15C are shown in Table v, along with the amounts in human milk. In comparison with

the milk diet, the biscuit has notably less lysine, methionine, threonine and valine. In comparison with human milk, there are still deficiencies of lysine, methionine and valine, but they are smaller for lysine and methionine, and there is no shortage of valine.

There is not much useful information at this time regarding the nitrogen partition in the urine. For several years, we have been interested in the possibility that urea excretion could be used to measure the extent of the deamination of the amino acids of dietary protein, and we hoped to find, in the balance experiments, some relation between the quantity and quality of protein intake and urea production. So far we have found none, and the only observation that might be helpful is that nine of the twenty-two children whose results are given in Table IV produced the same percentage of their nitrogen intake as urea nitrogen from both the diets they received. From one child to another, however, the percentages differed widely, and it seemed obvious that the amounts of urea produced were not directly related to the nitrogen intake. The urea clearances were not altered in any consistent manner when the diets were changed. The greater excretion of ammonia by the children who had the milk diets was expected because those diets contained more phosphate than the biscuit diets, but the greater excretion of amino nitrogen was less easy to understand. It would be helpful to know what the excreted amino acids were, and whether one kind of diet or another caused more or less of the essential amino acids to appear. Although it might not be possible to correlate the efficiency for synthesis of a mixture of dietary proteins with an apparent waste of amino acids, some idea might be gained of the conditions under which maximum efficiency is achieved.

The percentages of fat absorbed, although a little lower than might be found in healthy European children, were still high. Except for the amount in the dried skim milk, which was probably negligible, all dietary fat was of vegetable origin. The good absorption was expected because we have included cottonseed oil in the diets of several hundred children who were being treated for kwashiorkor, and even

in the most severe cases, and in the first days when the children were most acutely ill, we have found no sign of intolerance.

We have found only one published account of work that is closely comparable to ours, that of Demaeyer and Vanderborght[16] who made a long series of balance experiments on eighteen African children in the Belgian Congo. The children were from three to seven years old, except for one who was twelve, and they had been trained to collect their own urines and stools. They were recovering from kwashiorkor, and had been treated for at least four weeks before the experiments began. They were given a basic diet that provided about 8 gm. protein daily, and supplements of either dried skim milk, a combination of peanuts and beans, peanut flour (the same as that we used for our biscuit 8HC) or soybean flour; the supplements supplied 60 to 90 per cent of the total protein intake. The milk produced higher levels of nitrogen retention than the other supplements, of which all three gave similar results, but in the range of nitrogen intakes of 600 to 900 mg./kg. body weight/day, the retentions were much higher than we found: for the milk they rose with the nitrogen intake from 280 to 500 mg./kg., and for the others they rose from 190 to 350 mg./kg. Since it is known that nitrogen retentions are high at the beginning of treatment (we have already mentioned some of our results that confirm this fact) but fall later, it might be argued that the Congolese children were less advanced than ours in convalescence or perhaps in nitrogen repletion, despite their longer period of treatment. On the other hand, the average percentages of nitrogen absorption in the Congolese children, uncorrected for endogenous nitrogen, were 82 for the milk diet, and 73 to 78 for the other diets. The corresponding average percentages for our balances were 88 for the milk diets, 77 for the C range of biscuit, and 81 for the U range. In our experiments, therefore, we had much lower retentions but higher absorptions. The percentage of absorbed nitrogen that was retained from our diets was accordingly much less. It was only 23 from the milk diet and 25 from biscuit 15U, whereas

it was 62 from the Congolese children's milk diet and 49 from their other diets.

It is difficult to reconcile the two sets of results and we can only point out that the amounts of nitrogen excreted by the Congolese children in their urine were smaller than those excreted by our children, and that in Macy's observations on healthy American children[14] the percentage of absorbed nitrogen that was retained was under 10.

SUMMARY

Balance experiments were performed on twenty-two African children who were convalescing after treatment for kwashiorkor. Each child was given two test diets. In the diets, the protein was derived either from milk alone, or from mixtures of peanuts, corn and wheat flours, and dried skim milk. The mixtures were made in two ways, the milk being cooked with the other ingredients or added raw later.

A mixture containing 15 per cent of the milk, uncooked, produced nitrogen retentions that were approximately the same as those produced by the milk diet. The mixture had given excellent results in the treatment of acute kwashiorkor.

The results are discussed in relation to the calorie:protein ratios, the percentage of dried skim milk, the availability of the lysine and the amino acid composition of the diets. They are also compared with the results of experiments on African children in the Belgian Congo.

A high percentage of the fat of all the diets was absorbed, confirming clinical experience. The ratio of fat to protein in the stools varied with the kind of diet; it was lowest when the milk diet was given.

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Diet Therapy

Planning the Low Calorie Diet

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HERE IN the United States many people are looking for painless reducing diets. Unfortunately there are none; in general the only persons who think there are are those who have never had to consume a calorically restricted diet. For all the hundreds of words devoted to the subject, there is no magic formula to be offered.

Before becoming concerned with details of a diet, consideration should be given to whether or not the overweight patient should be subjected to a reducing regimen. Not every obese patient is a candidate for weight reduction by dietery means. Evidence for such a conclusion may be found in the high rate of failure reported by various clinics for weight reduction,[1-5] the high recurrence of obesity uncovered in follow-up studies,[6-11] and the emotional symptoms and even mental breakdowns reported during the period of weight reduction.[3,12,13] No one questions the desirability of having an obese person lose weight if it can be done without causing him any emotional trauma. However, in most cases in which emotional factors are a part of the etiology of the obesity, there seems to be little hope for successful weight reduction over any period of time, unless some psychiatric help is first given, otherwise the experience may be a frustrating and guilt-producing one for the patient. In addition, countless hours may be saved both by the therapist and patient if an attempt is made to evaluate the suitability of the patient for weight reduction before it is undertaken. How the physician makes this evaluation is not easy to say.

The physician needs to consider the personality of the patient and his environmental and emotional circumstances at the moment. For what purpose does the patient appear to be using food? Is it more than physical nourishment to him? What does the patient hope to gain from weight reduction? Just weight loss (a realistic goal) or does he see it as some magic tool to make possible unrealistic aspirations? Are his life circumstances at the moment ones which make it likely for him to be able to accept changes in his eating and activity behavior? All these factors need to be considered. A thoughtful evaluation of each patient in terms of weight reduction is needed rather than automatic referral to a calorically restricted diet.

In our experience the patient for whom weight reduction by dietary means is most likely to be successful is one who meets the following criteria: (1) has a good emotional adjustment; (2) is in the early stages of obesity; (3) obesity developed in adult life rather than in childhood; (4) has no previous history of attempts at weight reduction with failure or with regaining of the weight lost; (5) has a reason meaningful to him for losing weight; and weight reduction is a realistic goal to the patient.

Given, then, an obese patient who is relatively stable emotionally, weight will be lost only when his caloric expenditure becomes greater than his caloric intake so that body fat may be burned. This caloric status can be achieved either by increased energy expenditure in the form of additional physical activity or by

reduced caloric intake in the form of food and drink, or a combination of both. In the obese patient without apparent emotional problems, lack of any sustained physical activity may be a prime factor in positive energy balance with its attendant fat accumulation. Even for weight maintenance, unless one wishes to keep a tight rein on food intake, too much stress cannot be placed on the need for more physical activity of the type which becomes a part of the daily pattern of living.

From a practical viewpoint much of the correction of caloric excesses in the already obese person must be brought about by means of a diet restricted in calories. As already stated, there is no magic formula for a painless low calorie diet. The only person for whom a low calorie diet is truly painless is the person who is already following such a diet; not the person who is gaining satisfaction from the consumption of a relative excess of calories.

So far, for practical purposes, we do not understand the mechanism or mechanisms for the control of appetite and hence cannot involve these in our diet planning. One of the prime requisites of a reducing diet should be its adequacy with regard to all nutritional needs except calories. It is obvious that there may be many kinds of nutritionally adequate low calorie diets. The one chosen will depend on the tastes and usual patterns of the person who is to consume it. With the exception of a limited number of people who must be ritualistic and dramatize their efforts at weight reduction (a group in my experience for whom one can predict very little long-time success) the less the character of the diet usually eaten is changed the better. All good diet therapy calls for adjusting the diet to the patient's usual patterns of eating in so far as may be consistent with therapeutic purposes. Such a procedure is the surest way of making a distasteful procedure more acceptable. Kurt Lewin, the famous German social psychologist has said that people like what they eat, rather than eat what they like.[14]

Therefore it can be said that any low caloric diet may be a good one for reducing purposes if it meets the following critieria: (1) The diet should satisfy all nutritional needs of the patient except calories. (2) It should be adapted as closely as possible to the dietary habits and tastes of the patient for whom it is intended. (3) It should protect the patient as much as possible from between-meal hunger and leave him with a sense of well-being and a minimum of fatigue. (4) The diet should be easy for the patient to obtain, whether at home or away, without making him feel "different." (5) It should be one which followed over a period of time retrains eating habits so that with suitable caloric additions, it may become a pattern for life-time eating.

The basis of a low calorie diet is the same as that of any well balanced normal diet (Table I). In general, nutritional adequacy for the adult will be assured if the diet includes each day: one pint of milk; one egg; two servings of meat, fish, poultry or substitute; four or five servings of fruits and vegetables, one of which is a green leafy or yellow vegetable and one of which is rich in ascorbic acid, such as citrus fruits or tomatoes; one or more servings of whole grain or enriched bread or cereal (the amount dependent on the caloric allowance and the previous dietary pattern of the patient); one or more teaspoons of table fat or oil as allowed by caloric level.

In my experience most patients who really adhere to a reducing diet prefer a simplified pattern which they can follow easily with a few simple directions rather than an elaborate prescription which calls for special foods and special preparations. Variety can be achieved by changes in the types of meats, fruits and vegetables used and in the combinations which can be made from the various foods allowed. The amount of calories may be varied by the amount of each food allowed.

The caloric level will vary with the rate at which the physician wants the patient to lose weight. Since the caloric equivalent of a pound of fatty tissue seems to be about 3,500 calories, a daily caloric deficit of 500 will be necessary for each pound of weight the patient is to lose per week. Except for special purposes the loss should not average more than 1 to 2 pounds per week. The actual caloric need of the patient varies according to his size, age and activity. Despite the enthusiasm of both

TABLE I

Sample High Protein Calorically Restricted Diets Based on "Normal" Diet Pattern

Food or Food Group*	Basic "Normal" Diet	Calorically Restricted Diets†			
		A	B	C	D
Milk exchanges	2	1 pint milk (skim)	1 pint milk (skim)	1 pint milk (whole)	1 pint milk (skim)
Eggs	1	2	2	2	1
Meat exchanges	2	6 oz. meat	8 oz. meat	8 oz. meat	8 oz. meat
Fruit and vegetable exchanges—at least one should be an ascorbic acid rich fruit; and one a carotene rich green leafy or yellow vegetable	4 to 5				
Citrus fruit		¹/₂ cup juice	¹/₂ cup juice	¹/₂ cup juice	¹/₂ cup juice
Vegetable group A (up to 1 cup)		X‡	X‡	X‡	X‡
Vegetable group B (¹/₂ cup = 1 serving)		2 servings	1 serving	1 serving	3 servings
Other fruit (fresh or unsweetened canned or frozen)		1 serving	2¹/₂ servings	2¹/₂ servings	2¹/₂ servings
Enriched or whole grain breadstuff or cereal (bread exchanges)	1 or more servings	1 serving	1 serving	1 serving	5 servings (one as potato)
Table fat or oils (fat exchanges)	1 or more teaspoons	. . .	1¹/₂ servings	2 servings	. . .
	Plus other calories sufficient to maintain desirable body weight	. . .	. . .	. . .	. . .

* For definition of exchange see reference 20 or 21.

† Composition (approximate):   Calories   Protein   Fat

                Diet A   1000   80   45

                Diet B   1200   90   60

                Diet C   1400   90   80

                Diet D   1400   95   45

‡ One serving of vegetable group A or B to be a green leafy or yellow vegetable.

patient and physician for removing excess weight quickly, our experience has shown that too low a caloric level is not desirable because the patient will supplement his intake as he chooses. We have obtained better results when the diet given was adequate to keep the patient reasonably comfortable. In such cases, if the patient is stable emotionally and personally motivated to lose weight, he is more apt to adhere to the prescribed regimen and lose weight. For women, the calorie prescription usually varies from 1,000 to 1,400, with the younger, more active women at the latter level. For men, 1,500 to 2,000 calories are frequently used, with 1,800 a common figure for men who have any physical activity. I prefer to start a patient at a fairly high caloric intake, try to get good cooperation and then adjust the caloric intake according to the weight loss of the patient.

There seems to be general agreement that a fairly high protein diet is more acceptable to the patient than one lower in protein.[15-19] The level usually suggested is 1 to 1.5 gm. per kg. of body weight. The high protein type of low caloric diet usually results in greater sense of well-being on the part of the patient, greater satisfaction, less between-meal hunger, less fatigue and a greater willingness to continue the diet. The reason for this is not clear al-

though many explanations have been offered.

Variation in the proportion of calories coming from fat and carbohydrate sources is one way in which a diet of a given caloric and protein level may be adjusted to the habits of the patient and also to the therapeutic plan of the physician.   Using the basic normal diet pattern outlined earlier, the fat level may be varied by the use of whole or skim milk, fat or lean meat, or the amount of table spreads or oils given.   The carbohydrate level can be varied easily by the amount of cereal and breadstuffs, and the type and quantity of fruits and vegetables prescribed (Table I).

An example of how a basic "normal" diet pattern may be modified to various levels of calorically restricted diets for women is given in Table I.   Since the food exchange method for simplified diet calculations has been widely accepted, the quantities and classification of foods in the table are given in terms of the various food exchanges, namely: milk, meat, fruit, vegetable (group A and group B), bread and fat.   For exchange possibilities within a food group and the quantities of each the reader is referred to either the original report[20] or Turner[21] for details.   Diets A, B and C are illustrative of the changes incurred in a high protein diet by increasing calories from 1,000 to 1,400.   The protein content at the 1,000 calorie level is approximately 80 gm.; at the 1,200 and 1,400 levels, 90 gm.   It is difficult to obtain 90 gm. of protein from the usual food sources in this type of diet and keep the caloric level as low as 1,000 calories.   The increase from 1,200 to 1,400 calories was accomplished by an increase in fat intake through the use of whole milk instead of skim milk, and slightly more table fat and oil.   Diet C is essentially the moderate fat, high protein diet which we have used in much of our work and is based on the formula developed by Ohlson.[22]   Diet D represents what can be accomplished with more or less the same caloric and protein levels but a lower fat diet. Here the calories from fat are reduced by the use of skim milk, only one egg and the elimination of table fats or oils.   The calories are

* Approximately: 1400 calories;   protein, 90 gm.; fat, 80 gm.;   carbohydrate, 80 gm.

TABLE II

A Suggested Meal Pattern of High Protein, Moderate Fat Type*

Breakfast
Citrus fruit juice: $^1/_2$ cup; or 1 small orange, or $^1/_2$ small grapefruit
Eggs: two
Bread (enriched or whole wheat):   1 thin slice
Butter or fortified margarine: 1 level teaspoon
Black coffee or tea: as desired

Lunch
Meat (or exchange):   4 oz.
Vegetable group A: up to 1 cup
Butter, margarine or oil: 1 teaspoon
Whole milk: 1 cup
Fruit, fresh or unsweetened: $^1/_2$ cup
Black tea or coffee: as desired

Dinner
Meat (or exchange):   4 oz.
Vegetable group B: $^1/_2$ cup
Fruit, fresh or unsweetened: $^1/_2$ cup
Whole milk: 1 cup
Black tea or coffee: as desired

Suggestions

Many kinds of meats, fruits and vegetables may be used to give variety
The foods listed may be used in various combinations to give variety

Meats
Weights do not include bone
Meats may be roasted, broiled, simmered, stewed, steamed or pan-broiled
No fat or flour should be used in preparation unless taken from the meal pattern

Vegetables
May be used cooked or raw
Use green leafy or yellow vegetables at least once a day if possible

Group A includes

Asparagus	Eggplant	Lettuce
String beans	Greens:	Mushrooms
Broccoli	Beet	Okra
Brussels sprouts	Swiss chard	Pepper
Cabbage	Collard	Radish
Cauliflower	Dandelion	Sauerkraut
Celery	Kale	Summer squash
Chicory	Mustard	Tomatoes
Cucumbers	Spinach	Water cress
Escarole	Turnip	

Group B includes

Beets	Peas, green	Squash, winter
Carrots	Pumpkin	Turnip
Onions	Rutabaga	

Fruit
Use only fresh fruit or unsweetened canned or frozen fruit
One citrus fruit or its equivalent in ascorbic acid content should be used each day

Use only foods listed in pattern
Avoid any additional butter, margarine, fats, grains, salad dressing, flour or sugar other than that indicated in pattern
Seasonings, spices, black coffee, black tea, fat-free bouillon and lemon may be used as wished unless physician advises otherwise

replaced from sources of carbohydrate by a marked increase in the use of bread exchanges (including cereals, crackers, grains and the higher carbohydrate vegetables such as potatoes) and in the number of servings of B group vegetables (beets, carrots, onions, peas, rutabaga, winter squash and turnips). Obviously the use of the lower fat level will give a bulkier but to some a less palatable diet.

We have worked with many diets, each of which has proved satisfactory for at least some patients. In our experience the diet which has come close to fulfilling the criteria listed for the greatest number of patients has been the high protein, moderate fat, low carbohydrate diet developed by Ohlson[22] and similar to diet C in Table I. Table II gives a suggested meal pattern of the high protein, moderate fat type (diet C) along with suggestions to the patient for the use of this pattern.

In my experience, if the person did not have at least a reasonable degree of emotional stability and some motivation meaningful to him to make weight reduction worth the struggle, no diet no matter how carefully concocted was successful in bringing about weight loss. But given the stability and motivation, the diet plan which took reasonable account of his usual food habits had a better chance of success. The "reasonable account" factor includes not only the kind of food, but also its use and distribution over the twenty-four-hour period. In most cases there are certain periods during the day or evening when a person seems to find it necessary to eat. It is wise to take these into account in planning the distribution of his low calorie diet.

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Reviews of Recent Books

Modern Nutrition in Health and Disease, 2nd edition, by Michael G. Wohl and Robert S. Goodhart. Lea & Febiger, Philadelphia, 1960, pp. 1152, $18.50.

This authoritative work, prepared under the direction of two recognized experts in the field of nutrition, has been revised with the assistance of fifty-nine collaborators, many of whom contributed to the first edition five years ago. Rapid advances have taken place in this science with the application of modern biochemical and physiologic technics to the problems of nutritional needs, cellular metabolism, food processing and many related aspects of the basic knowledge of nutrition and their relation to the treatment of disease.

The initial section of the text deals with normal nutrition. The important concept of body composition and the analysis of its metabolically distinct components providing more precise norms for guidance in nutrition is elaborated by Keys. Intermediary metabolisms of protein, fat and carbohydrate are discussed by Geiger, Alfin-Slater, Denel, Soskin and Levine. These respective chapters provide a clear understanding of the mechanisms of absorption, digestion and utilization of foods. Accessory food factors, including minerals and vitamins, have been the subject of extensive investigation in recent years; these data are brought into perspective by contributors intimately familiar with these important nutriments.

The second section establishes the foundations for the nutritional management of disease. The orientation of these chapters uniquely correlates clinical findings with dietary patterns necessary to improve the course of a wide variety of illnesses from infection to allergy. Representative diets are included illustrating the types of foods employed in the management of renal, hepatic, cardiovascular, gastrointestinal and many other disorders. There are several omissions in this section, including the role of gluten in the pathogenesis of sprue, and the dietary management of phenylketonuria and other genetically determined diseases. A number of omissions were found in the index in attempting to refer to specific subjects included in the text.

The final section of the book deals with nutrition in periods of physiological stress. There is much valuable material found in these chapters relating to the diet in pregnancy, infancy and adolescence, aging, working and emergency feeding. The entire volume is an important source of new and detailed information on fundamental knowledge of nutrition and its application to the treatment and prevention of disease.
C. R. SHUMAN

The Heinz Handbook of Nutrition: A Comprehensive Treatise on Nutrition in Health and Diseases, edited by B. T. Burton. McGraw-Hill Book Co., Inc., New York, 1959, pp. 439, $5.75.

This handbook is intended to serve as a reference manual for those interested in the practical aspects of nutrition. The fundamental principles of biochemistry and physiology are reviewed in a clear and concise manner. The main part of the book deals with the various aspects of nutrition in health, such as nutritional requirements, nutrition in pregnancy, lactation, childhood and old age, and with the therapeutic and preventive aspects of diet in relation to a variety of diseases.

Much material is covered in this book. For this reason only well established concepts are presented. Since the book was not written for students but for experienced workers in nutrition this is not a serious shortcoming. There is, however, a lack of citations to the literature. The value of the book would be much enhanced if sources of material were indicated. Despite these shortcomings, the book can be recommended as a reference manual for those interested in diet therapy.
M. W. BATES

Food Values in Shares and Weights, 2nd edition, by Clara Mae Taylor. The Macmillan Co., New York, 1959, pp. 116, $4.00.

This is a second edition of a book which has been widely used as a reference for food values. Food is given in both shares and weights along with their caloric, protein, fat, carbohydrate, calcium, iron, vitamin A, thiamine, riboflavin and ascorbic acid contents. Recommended daily requirements of food and effects of cooking on essential nutrients are discussed in an introductory section. The appendix contains practical tables on optimum weight and height relations and a method for recording physical growth. A major deficiency is the absence of any information on sodium and potassium contents of foods. This knowledge is so important to any person interested in diets that its omission greatly limits the usefulness of this book.
C. M. LEEVY

Significant Trends in Medical Research. Ciba Foundation Tenth Anniversary Symposium, edited by G. E. W. Wolstenholme, Cecilia M. O'Connor and Maeve O'Connor. Little, Brown & Co., Boston, 1960, pp. 356, $9.50.

There are few books that are of equal interest to all groups of medical scientists. This symposium, however, achieves this distinction. Some thirty eminent scientists from all over the world—seven of them Nobel

Laureates—met to discuss developments of the past ten years which they believed would be most significant in the next decade. The topics range from molecular structure (Pauling) to American medical research (Shannon). Readers of the Journal will be especially interested in the discussion of hormones (F. G. Young), metabolism (Best) and clinical nutrition (Brock). Of equal interest is Pickering's discussion of "the quantitative approach to disease" and Burnet's speculation on the population dynamics of body cells.

The outstanding feature of the book is "the union between entirely different minds in different disciplines and from different countries." This is a heartening and portentous sign.   S. O. W.

Infant Foods and Feeding Practice, by Herman F. Meyer. Charles C Thomas, Springfield, Ill., 1960, pp. 332, $9.75.

The title of this book accurately describes its contents. The listing and classification of the multitude of milks and milk substitutes available for infant feeding is valuable in that it makes readily available the information needed to enable the physician to use any product judiciously. Furthermore, by assigning the various products to categories, it becomes possible to make meaningful comparisons between different types of feedings. The author has accomplished his expressed aim not to be didactic in his recommendations of one product over another. However, this reviewer believes that a little more dogmatism would be useful particularly for the person uninitiated in infant feeding, and this book will be of value primarily to the uninitiated.

The sections on clinical problems of breast or bottle feeding, time of introduction of solids and attitudes toward feeding, while presenting both sides of these questions, still convey a balanced, middle-of-the-road approach. There will be disagreement with the author's interpretation of colic; however, his obviously genuine humility and honesty make it likely that he anticipated such a reaction.

In a book of this nature, it would be hoped that more of the fundamental aspects of nutrition would be covered, particularly with reference to newer information. The only reference to vitamin E, for example, is a notation designating the relation between units and milligrams. Nevertheless, as a clinical guide to the feeding of well infants, this book should be useful.
                                                            C. N. C.

BOOKS RECEIVED

Books received for review by THE AMERICAN JOURNAL OF CLINICAL NUTRITION are acknowledged in this column. As far as practicable, those of special interest are selected, as space permits, for extensive review.

The Inspection of Food. A Handbook for Students of Public Health, Agriculture and Meat Technology, second edition, by Horace Thornton. Bailliére, Tindall & Cox, London, 1960 (Williams & Wilkins Company, American agents), pp. 213, $3.75.

The Chemistry of Lipids in Health and Disease, by H. K. King. Charles C Thomas, Springfield, 1960, pp. 104, $3.75.

Internal Medicine: Abstracts of Soviet Medicine, Part B. Exerpta Medica Foundation, 1960.

Clinical Endocrinology. I, edited by Edwin B. Astwood. Grune & Stratton, New York, 1960, pp. 724, $18.75.

Medical Surveys and Clinical Trials. Some Methods and Applications of Group Research in Medicine, edited by L. J. Witts. Oxford University Press, New York, 1959, pp. 325, $8.00.

Modern Scientific Aspects of Neurology, edited by John N. Cumings. Edward Arnold Ltd., London, 1960 (Williams & Wilkins, American agents), pp. 360, $13.00.

Food Becomes You, by Ruth M. Leverton. . Iowa State University Press, Ames, 1960, pp. 198, $3.50.

Ciba Foundation Symposium on Cellular Aspects of Immunity, edited by G. E. W. Wolstenholme. Little, Brown & Co., Boston, 1960, pp. 495, $10.50.

Food For Space Travel, by Lt.Col. Albert A. Taylor, Beatrice Finkelstein and Robert E. Hayes. Armed Services Technical Information Agency, Arlington, 1960, pp. 66.

Klinische Physiologies. Actuelle Probleme in Übersichten, (Volume I, Number 2) edited by W. A. Müller. Georg Thieme Verlag, Stuttgart, 1960 (Intercontinental Medical Book Corporation, American agent), pp. 84, DM 18 ($4.30).

Oxymetrie. Theorie und Klinische Anwendung, edited by Kurt Kramer. Georg Thieme Verlag, Stuttgart, 1960 (Intercontinental Medical Book Corporation, American agent), pp. 206, DM 39.60 ($9.40).

Endemic Goitre. World Health Organization, Monograph Series No. 44. World Health Organization. Geneva, 1960, pp. 471, $8.00.

Biochemical Values in Clinical Medicine. (The Results Following Pathological or Psychological Change), by Robert Duncan Eastham. Willams & Wilkins Co., Baltimore, 1960, pp. 144, $3.75.

Diabetic Care in Pictures, third edition, by Helen and Joseph Rosenthal. J. B. Lippincott Company, Philadelphia, 1960, pp. 237, $4.50.

The Misrepresentation of Arthritis Drugs and Devices in the United States, by Ruth Walrod. Arthritis and Rheumatism Foundation, New York, 1960, pp. 168, $3.50.

Cirrhosis of the Liver, by Martin S. Kleckner, Jr. Charles C Thomas, Springfield, 1960, pp. 729, $24.50.

British Medical Bulletin: Insulin (September, 1960). The British Council, London, pp. 264, $3.25.

Ciba Foundation Symposium. Congenital Malformation, edited by G. E. W. Wolstenholme and Cecilia M. O'Connor. Little, Brown & Co., Boston, 1960, pp. 308, $9.00.

Ciba Foundation Colloquia on Endocrinology, Volume XIII. Human Pituitary Hormones, edited by G. E. W. Wolstenholme and Cecilia M. O'Connor. Little, Brown & Co., Boston, 1960, pp. 336, $9.50.

Abstracts of Current Literature

CHARLES R. SHUMAN, M.D., EDITOR

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VITAMIN E

The most potent vitamin E compound is alpha-to-copherol. The synthetic alpha-tocopherol acetate is the standard for vitamin E activity, 1 mg. representing the international unit of the latter. The physiologic action in man is not known but in animals a variety of changes are observed in vitamin E deficiency states. The administration of large amounts of unsaturated fats has been shown to reduce vitamin E activity in man and in experimental animals. In animals, this deficiency is a cause of encephalomalacia; it is possible that the same effect may be observed in man.

Relationship Between Vitamin E in the Free and Acetate Form Present in the Plasma After Parenteral Administration of Tocopherol Acetate. G. Rindi and V. Perri. *Internat. Ztschr. Vitaminforsch.*, 28: 274, 1958.

A single dose of 300 mg. of DL-alpha-tocopherol acetate in aqueous emulsion, administered intramuscularly to a human subject, engenders a rapid increase of the plasma esterified tocopherol content which continues until the eighth hour following the injection, and drops thereafter until the forty-eighth hour.

The free tocopherol instead increases manifestly only from the eighth hour up to the thirty-second hour, and remains unchanged at the levels reached up to the forty-eighth hour.

It is concluded that aqueous emulsions of alpha-tocopherol are a much better form of parenteral application of vitamin E than are oily solutions as they produced a significant rise of the serum level of vitamin E.

AUTHORS

Ineffectiveness of Factor 3—Action Selenium Compounds in Resorption-Gestation Bioassay for Vitamin E. P. L. Harris, M. I. Ludwig and K. Schwarz. *Proc. Soc. Exper. Biol. & Med.*, 97: 686, 1958.

Purified factor 3, which is characterized by an organic substance containing selenium, prevents certain deficiencies attributed to lack of vitamin E, such as degeneration and necrosis of liver, heart, muscle and kidney particularly of rodents, and exudative diathesis of birds. Since resorption of fetuses during pregnancy is thought to be specifically due to vitamin E deficiency, a gestation bioassay was made in rats in order to determine whether or not the selenites might counteract the lack of tocopherol. Weanling female rats were fed a diet low in vitamin E; when they had reached a weight of 150 gm. they were mated with males fed a stock diet and given supplements of either tocopherol or high doses of test substances such as selenocystin, sodium selenite or selenium acid on each of five consecutive days of pregnancy. The animals were killed on the twentieth day of gestation. While tocopherol counteracted the harmful effect of the deficient diet, no effects were observed after administration of the selenium compounds. Thus factor 3-selenium is not identical with tocopherol, although both are essential for a complete diet.

M. SILBERBERG

Vitamin E deficiency in animals produces degenerative changes of germinal epithelium; in females, there is an increase in stillbirths and a resorption of fetuses. Degeneration of striated muscles and cardiac muscle with creatinuria occurs with prolonged deprivation. The dystrophic changes observed in animals have been compared to muscular dystrophy in man.

Effect of Vitamin E Deficiency on Creatine Phosphokinase of Heart and Skeletal Muscle. W. O. Read and S. Nehorayan. *Am. J. Physiol.*, 196: 1286, 1959.

A quantitative determination of creatine phosphokinase activity of heart and skeletal muscle has been

made in early and severe vitamin E deficiency in the rabbit. This study revealed that early vitamin E deficiency resulted in an increase in creatine phosphokinase activity of skeletal muscle but decreased the enzymatic activity of the heart. Severe vitamin E deficiency resulted in a decrease in creatine phosphokinase activity of both skeletal and heart muscle. 17-Hydroxycorticosterone, administered in small doses, resulted in an increase in enzymatic activity of skeletal muscle but no change in heart muscle. Large doses of 17-hydroxycorticosterone caused a decrease in the creatine phosphokinase activity of both heart and skeletal muscle. Normal male rabbits exhibited a lower enzymatic activity than female animals, a difference which, in part, was due to testosterone.   AUTHORS

Effects of Selenium and Vitamin E on White Muscle Disease. O. H. Muth, J. E. Oldfield, L. F. Remmert and J. R. Schubert. *Science*, 128: 1090, 1958.

White muscle disease is a myopathy in lambs and calves which results when legumes from certain areas are fed during gestation. When 100 International Units of alpha-tocopherol were fed daily to the animals on a basal ration, sixteen of the twenty lambs were affected with white muscle disease. However, when 0.1 part of selenium per million was given, it appeared that selenium had a definite protective pharmacodynamic effect in this disease under the conditions of the experiment. As the authors suggest, a more comprehensive and critical investigation should be made of the role of this element in white muscle disease and other myopathies in animals and in man.   S. O. WAIFE

Effect of Short-Term Vitamin E Deficiency on Guinea Pig Skeletal Muscle Myoglobin. A. D. Bender, D. D. Schottelius and B. A. Schottelius. *Am. J. Physiol.*, 197: 491, 1959.

Myoglobin concentration was determined in gastrocnemius and masseter muscles of guinea pigs maintained up to fifteen days on vitamin E-deficient and vitamin E-supplemented diets. A statistically significant increase in myoglobin was noted in muscles of animals on the deficient diet for fifteen days. That the increase was real and not apparent was attested by studies of total nitrogen, non-collagen nitrogen, percentage of solids and muscle wet weight, all of which were the same in control and experimental muscles. Histological sections and creatine excretion studies confirmed the impression of mild, incipient nutritional dystrophy.   AUTHORS

Serum Proteins, Lipoproteins and Glycoproteins in Muscular Dystrophy of Vitamin E Deficiency. H. Oppenheimer, S. Shulman, S. Roberts and A. T. Milhorat. *Proc. Soc. Exper. Biol. & Med.*, 97: 882, 1958.

An investigation was made to determine whether or not the changes in serum lipid patterns known to be produced in animals by vitamin E deficiency are associated with changes in serum proteins. Rabbits were fed a vitamin E-deficient diet from which all traces of the vitamin had been removed by oxidation with ferric chloride. Control animals received the same diet with supplements of DL-alpha-tocopherol, 6 to 7 mg. in oil, twice weekly. Similar doses were used to treat established deficiency which was manifest by biochemical changes and muscular dystrophy.

Vitamin E-deficient animals showed an increase in the beta-lipoprotein and a decrease of the alpha-lipoprotein level in the serum with parallel changes in cholesterol and phospholipid concentrations. There was an over-all increase in the serum cholesterol and phospholipid levels. The increase in the free cholesterol was greater than that of the esterified fraction. The total serum protein level remained constant but there was a small decrease in the albumin and an increase in beta-globulin. The concentration of serum protein-bound carbohydrate increased slightly. All these changes, as well as the increased ratio of urinary creatine to creatinine which accompanied the muscular dystrophy, were reversed by the administration of DL-alpha-tocopherol.   G. WALKER

Favorable reports upon treatment of vascular insufficiency due to atherosclerosis with vitamin E have emerged sporadically for the past decade. The failure to confirm these reports by other investigators has dampened enthusiasm for this form of therapy. The following study indicates that further studies are required.

Treatment of Intermittent Claudication with Vitamin E. P. D. Livingstone and C. Jones. *Lancet*, 2: 602, 1958.

There is no agreement about the value of vitamin E in cases of intermittent claudication, and judgment is often impossible because of the difficulty of grading and classifying cases and the tendency for the condition to improve spontaneously in some cases.

Forty males without diabetes, who had had this disability for at least five years, were divided into two groups with approximately the same age distribution and the same grading of the condition by Boyd's classification. One group was given 600 mg. of vitamin E daily for forty weeks, the other group was given dummy tablets.

Three of each group dropped out of the trial; of the remaining seventeen taking vitamin E, thirteen felt great improvement; this was confirmed by exercise tolerance tests. In the control group only two of the seventeen men showed subjective and objective improvement; one recovered completely for no obvious reason, the other had popliteal aneurysms unknown at the time of selection.

Two important points emerge from this trial: (1) large doses of the vitamin are necessary, and (2) no improvement may occur for as long as several months

after treatment begins.   The improvement seemed to be lasting.   F. E. HYTTEN

The following contributions seem to indicate that vitamin E may function as a co-factor in several biochemical reactions.   Whether these effects are the result of its well known antioxidant activity or other unknown actions remains to be determined.

Vitamin E Deficiency in the Monkey.   III.   The Metabolism of Sodium Formate-C[14].   J. S. Dinning and P. L. Day.   *J. Biol. Chem.*, 233: 240, 1958.

Nutritional muscular dystrophy developed in rhesus monkeys in ten months when kept on a tocopherol-deficient diet.   Nucleic acids isolated from bone marrow and skeletal muscle from tocopherol-deficient monkeys injected with sodium formate-C[14] incorporated greater amounts of radioactive carbon than the same nucleic acids from control animals.   Resupplementation of the animals for three months following a ten-month depletion period resulted in a complete reversal of this lesion in bone marrow, but only a partial reversal in skeletal muscle.   The rate of uptake of formate-C[14] into desoxyribonucleic acid of bone marrow was elevated sixfold in vitamin E-deficient monkeys, but was restored to normal by resupplementation.   Considerable increases were also seen in the incorporation of labeled formate into the acid-soluble purines, nucleic acid purines and creatine of skeletal muscle from deficient animals.   From these results, as well as from previous studies in rats and rabbits, the authors claim that vitamin E has a role in regulating nucleic acid metabolism.
                                                    M. K. HORWITT

The Role of Lipides in Electron Transport.   IV Tocopherol as a Specific Cofactor of Mammalian Cytochrome c Reductase.   K. O. Donaldson, A. Nason and R. H. Garrett.   *J. Biol. Chem.*, 233: 572, 1958.

The experiments described in this paper indicate that the lipide co-factor of cytochrome c (glycol monopalmitate, -oleate and -stearate) isolated from bovine heart muscle acts by releasing endogenous vitamin E to "active sites" on the enzyme.   Extractions of cytochrome c reductase with iso-octane causes a 90 per cent reduction in the enzyme activity while removing only 10 per cent of the total vitamin E.   This amount of vitamin E is insufficient to restore the activity of the enzyme.   However, several natural and synthetic lipides as well as the heart co-factor were capable of restoring enzyme activity.   The same lipide substances which restored activity to the extracted fresh enzyme were also capable of increasing the amount of tocopherol removed from the enzyme by iso-octane extractions, suggesting that these substances mobilize the endogenous tocopherol.   When the enzyme was aged and extracted, thereby removing a much greater amount of endogenous tocopherol, activity was restored specifically by tocopherol, while the lipide co-factor and other lipides which were capable of reactivating the fresh

extracted enzyme were now ineffective.   The same lipides were now inactive in potentiating the removal of tocopherol from the aged tocopherol-specific enzyme. Vitamin E, therefore, appears to be a co-factor of cytochrome c reductase.   M. K. HORWITT

Inter-relationship Between α-Tocopherol (Vitamin E) and 5-Hydroxytryptamine (Serotonin).   A. Meyer. *Internat. Rev. Vitamin Res.*, 29: 77, 1958.

Contrary to certain other views, experimental evidence indicates that vitamin E exerts not only a general non-specific antioxidant effect, but also a specific effect as a vitamin.   It appears, like tyroxine, the substance promoting basic metabolic exchanges, to have a protective action on 5-hydroxytryptamine (serotonin) and so to prevent its premature destruction.   AUTHOR

The Curative Action of α Tocopherol and of Protein Upon the Incisor Teeth of Vitamin E-Depleted Rats. J. T. Irving.   *J. Dent. Res.*, 37: 732, 1958.

The incisors of young rats maintained on a vitamin E-deficient diet for thirty days showed the typical degenerative changes previously reported as attributable to vitamin E deficiency.   All incisor teeth observed grossly were white and, upon histologic study, the dialuric acid test was found to be positive with a practically complete or complete absence of iron in the ameloblasts. Fibrous tissue containing large numbers of macrophages replaced the ameloblasts.   In rats which received a daily supplement of 3 mg. of alpha-tocopherol throughout the experiment, the incisors remained the normal orange-yellow color and were normal histologically with negative dialuric acid tests.

In rats that were fed 3 mg. of alpha-tocopherol for varying periods from ten to eighty days after a preliminary vitamin E-depletion period of thirty to forty days, the first sign of recovery was a reappearance of iron in the ameloblasts about ten days after therapy was begun.   Macrophages decreased in number as the period of therapy increased.   The first appearance of the yellow pigment at the gingival margin occurred between twenty-nine and sixty-four days after therapy was begun.   The dialuric acid test was faintly positive after ten days of therapy but was consistently negative thereafter. In a fourth group of rats, the protein content of the diet was increased from 9.3 to 18.8 per cent after the usual thirty- to forty-day vitamin E depletion period.   The reparative results of the protein increase were virtually identical with those that had been seen in the alpha-tocopherol treated rats except that the dialuric acid test remained positive throughout the entire experimental period.

The discussion contains interesting comments on the reparative process and possible reasons for the prolonged repair period required by the enamel organ and the role of protein in substitution for vitamin E.
                                                    J. H. SHAW

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(ab.) = Abstract   (E) = Editorial

Clinical
Nutrition

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2

THE AMERICAN JOURNAL OF Clinical Nutrition

CONTENTS

B-vitamins or ascorbic acid

saturation doses – the hard way!

Each of these food portions contains a saturation dose of one of the water-soluble B vitamins or C. The easy way to provide such quantities of these vitamins with speed, safety and economy is to prescribe Allbee with C. Recommended in pregnancy, deficiency states, digestive dysfunction and convalescence.

In each Allbee with C:		As much as:*
Thiamine mononitrate (B_1)	15 mg.	6.9 lbs. of fried bacon
Riboflavin (B_2)	10 mg.	31½ ozs. of liverwurst
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Calcium pantothenate	10 mg.	¼ lb. of fried beef liver
Ascorbic acid (Vitamin C)	**250 mg.**	¾ lb. of cooked broccoli

*These common foods are among the richest sources of B vitamins and ascorbic acid. H. A. Wooster, Jr., **Nutritional Data**, 2nd Ed., Pittsburgh, 1954.

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Robins

A. H. ROBINS COMPANY, INC.
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CONTENTS *continued—November–December 1960*

A well-balanced moderate low-fat breakfast for teen-age girls

Teen-age girls comprise the most poorly fed group in our population today according to nutrition researchers. During this critical growth period, well over half of them skip or skimp on breakfast, the most important meal of the day. As a service to those advising teen-age girls and their parents, this *well-balanced, moderate low-fat basic cereal and milk breakfast* shown in the chart below merits consideration. Its moderate low-fat content of 10.9 gm.

provides 20 per cent of the total calories. This is in keeping with the modern trend toward a moderate reduction of dietary fat for all ages. For "Girls, 13 to 15 years," it is *well-balanced* and provides about one-fourth of the recommended daily dietary allowances! The Iowa Breakfast Studies demonstrated that *a basic cereal and milk breakfast was nutritionally efficient* for the young and old alike.

Recommended Daily Dietary Allowances* and the Nutritional Contribution of a Basic Cereal and Milk Moderate Low-Fat Breakfast

> *Menu:* *Orange Juice—4 oz.;*
> *Cereal, dry weight—1 oz.;*
> *Whole Milk—4 oz.; Sugar—1 teaspoon;*
> *Toast (white, enriched)—2 slices;*
> *Butter—5 gm. (about 1 teaspoon);.*
> *Nonfat Milk—8 oz.*

Nutrients	Calories	Protein	Calcium	Iron	Vitamin A	Thiamine	Riboflavin	Niacin equiv.	Ascorbic Acid
Totals supplied by Basic Breakfast	503	20.9 gm.	0.532 gm.	2.7 mg.	588 I.U.	0.46 mg.	0.80 mg.	7.36 mg.	65.5 mg.
Recommended Dietary Allowances—Girls, 13 to 15 Years (49 kg.—108 lb.)	2600	80 gm.	1.3 gm.	15 mg.	5000 I.U.	1.3 mg.	2.0 mg.	17 mg.	80 mg.
Percentage Contributed by Basic Breakfast	19.3%	26.1%	40.9%	18.0%	11.8%	35.4%	40.0%	43.3%	81.9%

Cereal Institute, Inc.: Breakfast Source Book.
Chicago: Cereal Institute, Inc., 1959.
Food & Nutrition Bd.: Recommended Dietary Allowances, Revised 1958.
Natl. Acad. Sci.—Natl. Research Council Publication 589, 1958.
Watt, B. K., and Merrill, A. L.: Composition of Foods—Raw,
Processed, Prepared. U.S.D.A. Agriculture Handbook No. 8, 1950.

**The allowance levels are intended to cover individual variations among most normal persons as they live in the United States under usual environmental stresses. Calorie allowances apply to individuals usually engaged in moderate physical activity. For office workers or others in sedentary occupations they are excessive. Adjustments must be made for variations in body size, age, physical activity, and environmental temperature.*

CEREAL INSTITUTE, INC.
135 South La Salle Street, Chicago 3

A research and educational endeavor devoted to the betterment of national nutrition

CONTENTS *continued—November–December 1960*

Diet Therapy

Special Departments

Erratum

We regret that on pages 722 and 723 of the September–October 1960 issue
(Vol. 8, No. 5), the illustrations for Figures 3 and 4 were inadvertently reversed.
All references to these figures in the text referred to the correct legends.

all this...

ESSENTIAL NUTRIENTS IN ONE EGG.

Protein............6.1 Gm.

Carbohydrate.......0.3 Gm.

Fat..............5.5 Gm.

(Unsaturated
Fatty Acids.......3.6 Gm.)

Also present, vitamins A,
D, E, K, B₁, B₂, B₆,
B₁₂, Pantothenic Acid,
Niacin, Folic Acid, Biotin,
and many essential minerals.

and only

77 calories

Few foods known to man provide a higher ratio of nutrient value to calories than do eggs.

The quality of egg protein is a standard against which the proteins of other foods are measured.

The cholesterol and fatty acid contents of two eggs—traditionally a widely preferred breakfast—fit well into the daily diet, even when lessened fat intake is recommended.

Because of their high nutrient value, their easy digestibility, compatibility, and nutritional complementation of other foods, eggs are included in the recommended dietary* for many conditions in which diet adjustment is indicated.

Eggs are listed in the daily recommendations of nourishing liquid, restricted fiber, low sodium, restricted purine, low-calorie, and many other diets.

*Recommended in the diet manuals of teaching institutions.

The nutritional statements made in this advertisement have been reviewed by the Council on Foods and Nutrition of the American Medical Association and found consistent with current authoritative medical opinion.

9

Inflammatory reaction
following stress!

In inflammation, either localized or generalized in nature, capillary damage — increased permeability, resulting in seepage of blood constituents into the tissues — is a uniform basic reaction resulting from injury or stressors of various types:

PHYSICAL: Trauma, surgery, overexertion, sprains

NUTRITIONAL: Malnutrition, toxins, pregnancy, growth

ENVIRONMENTAL: Temperature, pressure, radiation, allergies

DISEASE STATES: Viral, bacterial, malignancies, endocrine

The role of the citrus bioflavonoids in the prevention or reversal of the inflammatory process is multiple through:

1. Maintenance of capillary integrity

2. In cellular metabolic processes, by potentiating corticosteroids, vitamins and essential nutrients, and by inhibition of hyaluronidase

3. Direct anti-inflammatory action

In the treatment of inflammatory conditions include the citrus bioflavonoids (Lemon Bioflavonoid Complex, Hesperidin Complex and Hesperidin Methyl Chalcone) as therapeutic adjuncts.
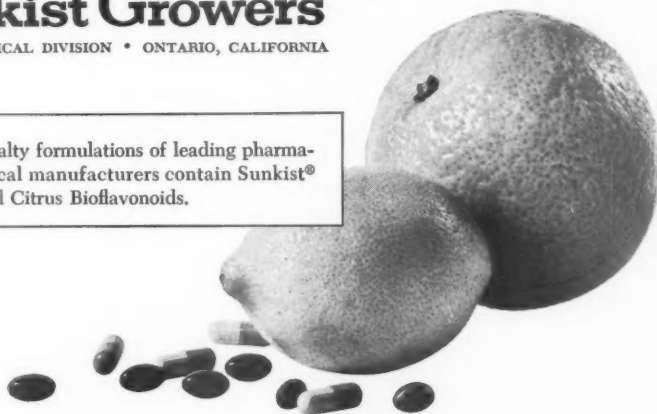
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PEANUT OIL	1.6
OLIVE OIL	.64
LARD	.26
BUTTER	.05

U. S. Dept. of Agriculture Home Economics Research Report No. 7 (1959)

L/S RATIO 1 2 3 4 5 6
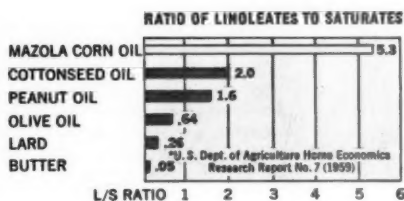
SELECTED BIBLIOGRAPHY—Leading authorities agree that substitution of polyunsaturated for saturated fats in the diet will lower serum cholesterol. The following references reflect current medical opinion. 1. Ahrens, E. H., *et al*, *J.A.M.A. 170* (18) 2198 (Aug. 29, 1959) 2. Louis N. Katz, Jeremiah Stamler and Ruth Pick, *Nutrition and Atherosclerosis* (1958), p. 108. 3. Boyer, P. A., *et al*, *J.A.M.A. 170* (3) 257 (May 16, 1959). 4. Jolliffe, N., *A. J. Clin. Nutrition 7*, 451 (1959). 5. Keys, A., *et al.*, *Circulation 19*, 201 (1959*)*.

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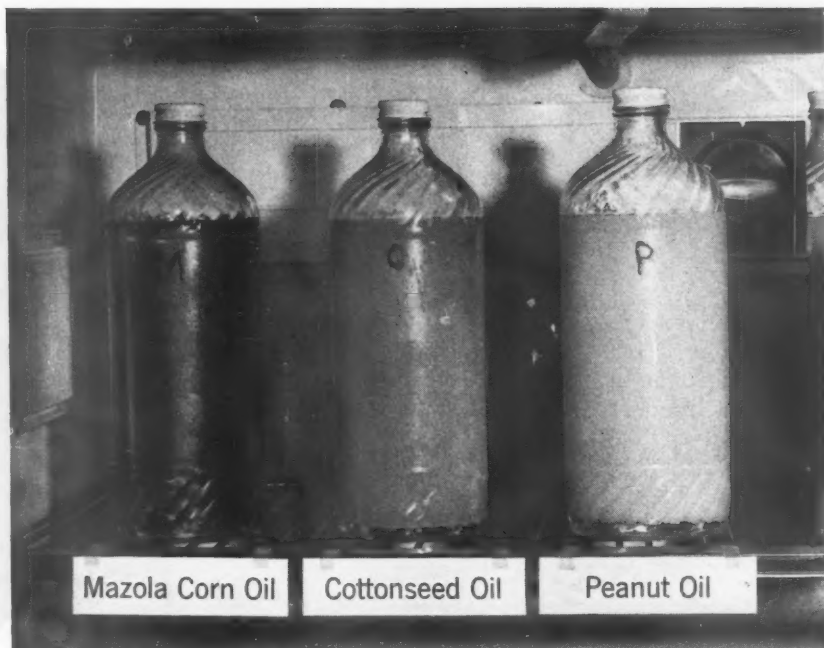
QUALITATIVE MEASURE OF L/S RATIO. After refrigeration for 96 hours at 33°F, peanut and cottonseed oils have clouded and precipitated. Only MAZOLA (left), having a far lower degree of saturation than other leading vegetable oils, remains unclouded.

13

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—pork provides an important amount of **other B vitamins**

—pork contributes significant amounts of the **essential minerals,** iron, copper and phosphorus, magnesium and potassium, supplied in a form that the body can use readily.

1. Leverton, R. M., and Odell, G. V.: The Nutritive Value of Cooked Meat, Oklahoma Agricultural Experiment Station, Oklahoma State University, Miscellaneous Publication MP-49, 1958.

PUT PORK IN ITS PROPER PLACE
. . . in the daily diet
. . . use to tempt jaded appetites
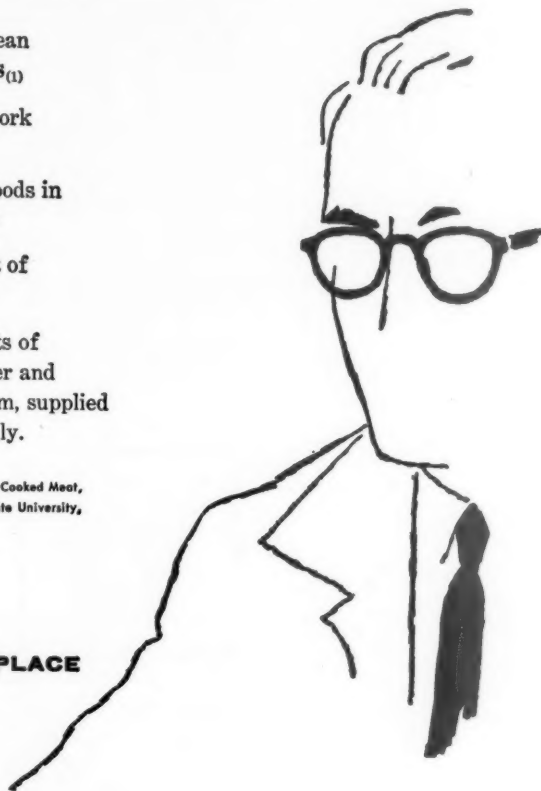
The nutritional statements made in this advertisement have been reviewed by the Council on Foods and Nutrition of the American Medical Association and found consistent with current authoritative medical opinion.

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15

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16

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17

she calls it "nervous indigestion"

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*The nutritional statements In "Weight Control . . . the Module Way" have been reviewed by the Council on Foods and Nutrition of the American Medical Association and found consistent with current, authoritative medical opinion.

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and whole wheat flour foods are listed among the "Essential Four" food groups set up by the U.S. Dept. of Agriculture's Institute of Home Economics. Diet selected from these foods provides ample protein, vitamins and minerals.

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The Roche Review
Of Enrichment Requirements

for Cereal Grain Foods in the United States

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	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Enriched BREAD or other *baked* products	1.1	1.8	0.7	1.6	10.0	15.0	8.0	12.5
Enriched FLOUR[1]	2.0	2.5	1.2	1.5	16.0	20.0	13.0	16.5
Enriched FARINA	2.0	2.5	1.2	1.5	16.0	20.0	13.0	*
Enriched MACARONI & NOODLE Products[2]	4.0	5.0	1.7	2.2	27.0	34.0	13.0	16.5
Enriched CORN MEALS	2.0	3.0	1.2	1.8	16.0	24.0	13.0	26.0
Enriched CORN GRITS[3]	2.0	3.0	1.2	1.8	16.0	24.0	13.0	26.0
Enriched Milled WHITE RICE[4]	2.0	4.0	1.2**	2.4**	16.0	32.0	13.0	26.0

* No maximum level established.

** The requirement for vitamin B_2 is optional pending further study and public hearings because of certain technical difficulties encountered in the application of this vitamin.

1 In enriched self-rising flour, calcium is also required between limits of 500-1500 mg. per pound.

2 Levels allow for 30-50% losses in kitchen procedure.

3 Levels must not fall below 85% of minimum figures after a specific test described in the Federal Standards of Identity.

4 The Standards state that the rice, after a rinsing test, must contain at least 85% of the minimum vitamin levels. The Governments of Puerto Rico and the Philippines also require this rinsing test. If the method of enrichment does not permit this rinsing requirement to be met, consumer size packages must bear the statement, "Do not rinse before or drain after cooking." Rice enriched by the Roche method will meet the rinsing test. The South Carolina law does not require a rinsing test on packages less than 50 pounds, as the rice in small packages is presumed to be sufficiently clean.

The maximum and minimum levels shown above for enriched bread, enriched flour, enriched farina, enriched macaroni, spaghetti and noodle products, enriched corn meal and corn grits and enriched rice are in accordance with Federal Standards of Identity or State laws. Act No. 183 of the Government of Puerto Rico requires the use of enriched flour for all products made wholly or in part of flour, including crackers, etc.

Brief, authoritative stories about the enrichment of many cereal grains have been gathered into a booklet which you may have for the asking. Just send your request for "The Vital Story of Cereal Grain Products" to the Department of Education, Fine Chemical Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey.

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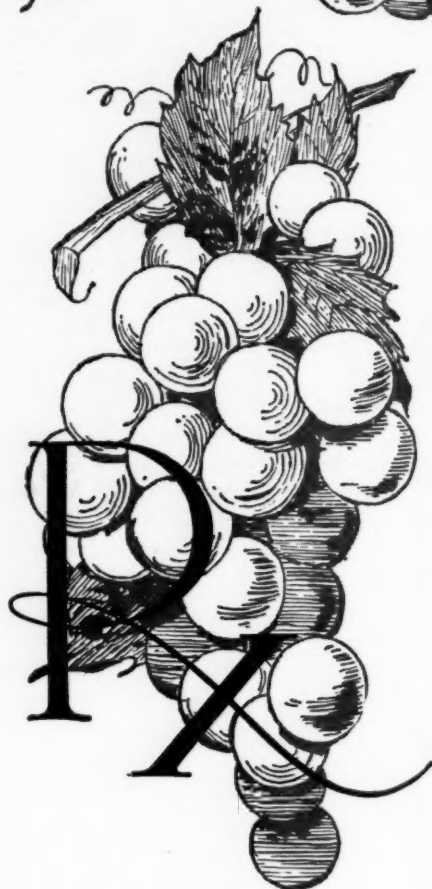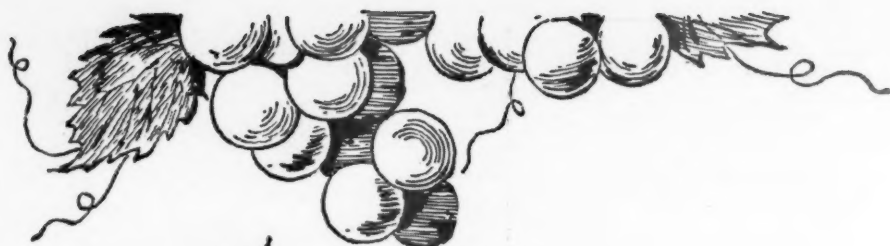
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*Floore, F. B.: Wine is Fine for Hospital Cookery, Mod. Hosp. 94:134 (June) 1960.

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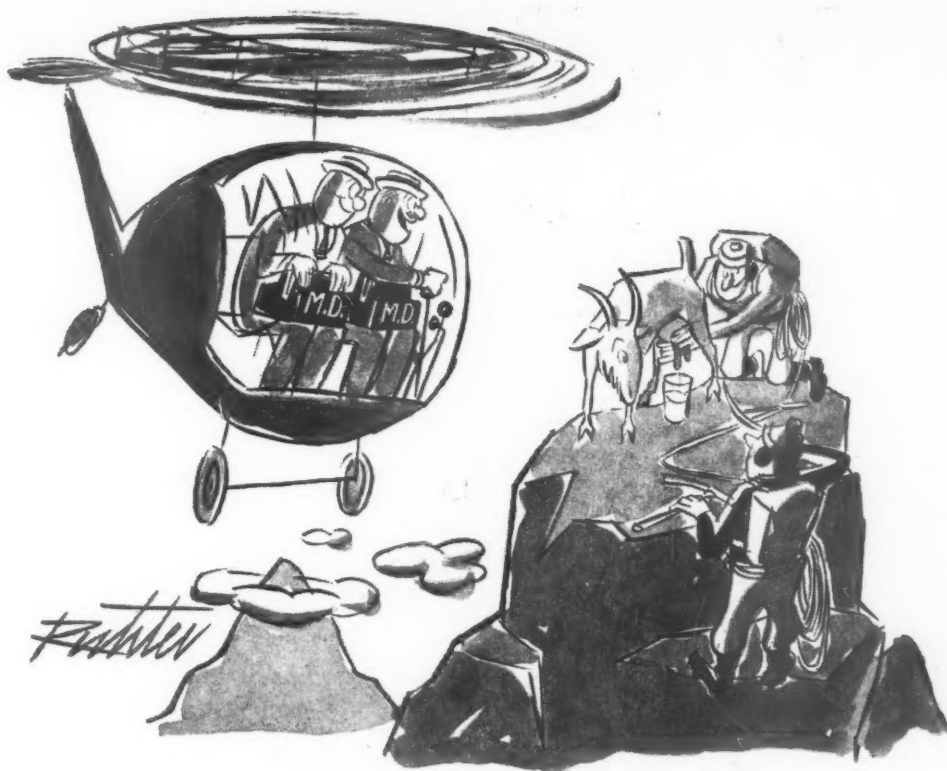
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